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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-882

Pharmacology Review(s)

MEMORANDUM

Nov. 1, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-882

I concur that the marketing application for Exjade® (deferasirox) Tablets for Oral Suspension may be approved based on review of the submitted pharmacology/toxicology data.



Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
11/1/2005 02:47:59 PM
PHARMACOLOGIST

**ADDENDUM TO PHARMACOLOGIST'S REVIEW OF NDA 21-882
Original Submission (000) dated April 29, 2005**

Reviewer Name: Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Date of Review: October 28, 2005

Drug: ICL-670 (Exjade®)

Category: Iron chelator

This addendum is to correct an error on page 268 of the review in the "Proposed Version" for labeling section "Carcinogenesis, Mutagenesis, Impairment of Fertility". This is reproduced below from the review of the original submission.

"Proposed Version: [

[]

Comments: The line 7 of the "Proposed Version" states "The dose of 200 and 300 mg/kg/day in males and females produced a systemic exposure to ICL670 ($AUC_{0.5-6h}$ of parent compound) of [] respectively." As per e-mail of Dr. Laniyonu (October 27, 2005), the sponsor stated that they could not verify the above values [] . These numbers [] are in error. Sponsor's provided values of 639 and 1100 $\mu\text{g}\cdot\text{hr}/\text{ml}$ are accurate and acceptable. However, it is to be noted here that the AUC values for mice were calculated based on 0.5 to 6 hour exposure ($AUC_{0.5-6h}$) whereas for human, the AUC values were calculated based on 24 hour drug exposure (AUC_{0-24h}). Therefore, in case of mice, AUC comparison does not appear to be appropriate in the absence of AUC_{0-24h} values for mice. In addition, for rats AUC vales were calculated based on 24 hour exposure, which allowed direct

comparison with human exposure. So, in order to maintain consistency between two species, we recommend using comparison based on body surface area. Moreover, body surface area was also used in other sections of the labeling for comparison with human data. So, we propose the following revised labeling for "Carcinogenesis, Mutagenesis, Impairment of Fertility".

Proposed Revised Version: "104-Week oral carcinogenicity study in Wistar rats has shown no evidence of carcinogenicity from ICL670 at doses up to 60 mg/kg/day (about 0.48 times the recommended human oral dose based on body surface area). A 26-Week oral carcinogenicity study in p53 (+/-) transgenic mice has shown no evidence of carcinogenicity from ICL670 at doses up to 200 mg/kg/day (about 0.81 times the recommended human oral dose based on body surface area) in males and 300 mg/kg/day (about 1.21 times the recommended human oral dose based on body surface area) in females.

Deferasirox was negative in the Ames test and chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests.

Deferasirox at oral doses up to 75 mg/kg/day (about 0.6 times the recommended human oral dose based on body surface area) was found to have no adverse effect on fertility and reproductive performance of male and female rats."

/s/

Tamal K. Chakraborti, Ph.D.
Pharmacologist

Date

Comment:

/s/

Jasti B. Choudary, B.V.Sc., Ph.D.,
Supervisory Pharmacologist

Date

cc:
NDA 21-882
HFD-180
HFD-160/CSO/PM/AKacuba/DMIHP
HFD-180/Dr. Choudary
HFD-180/Dr. Chakraborti
HFD-160/Dr. Lanionu
HFD-160/Dr. Chopra

R/D Init. J Choudary: 10/28/05

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this page is the manifestation of the electronic signature.**

/s/

Tamal Chakraborti
10/28/2005 12:18:08 PM
PHARMACOLOGIST

Jasti Choudary
10/28/2005 12:23:04 PM
PHARMACOLOGIST

NDA 21, 882

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA Number: 21-882

Review Number: 000

Sequence Number/Date/Type of submission: 000/April 29, 2005/Initial

Information to Sponsor: Yes () No ()

Sponsor: Novartis Pharmaceuticals Corporation, East Hanover, NJ

Manufacturer for Drug Substance:

1. Novartis Pharma AG, Basel, Switzerland
2. Novartis Pharma AG, Pratteln, Switzerland

Reviewer Name: Tamal K. Chakraborti, Ph.D.

Division Name: Gastrointestinal & Coagulation Drug Products

HFD #: 180

Review Completion Date: September 8, 2005

Drug:

Trade Name: Exjade

Generic Name: Deferasirox

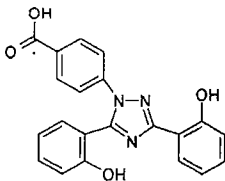
Code Name: ICL670 (ICL670A, CGP72670, PN3002862)

Chemical Name: 4-[3,5-Bis-(hydroxy-phenyl)-[1,2,4]-triazol-1-yl]benzoic acid

CAS Registry Number: 201530-41-8

Molecular Formula/Molecular Weight: C₂₁H₁₅N₃O₄/373.4

Structure:



Relevant IND: IND 58,554 (ICL670, Novartis, HFD-180)

Drug Class: Iron chelator

Indication: ICL670 is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis).

Clinical Formulation: ICL670 125, 250 or 500 mg Oral Dispersible Tablets. Each tablet contains standard excipients (lactose monohydrate, crospovidone, povidone, sodium lauryl sulfate, microcrystalline cellulose, silicon dioxide and magnesium stearate).

Route of Administration: Oral (dispersible tablet)

Proposed Use: ICL670 is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis).

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

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**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Rat Carcinogenicity Study Results**

P/T REVIEWER: Tamal K. Chakraborti, Ph.D.

DATE:

NDA: 21-882

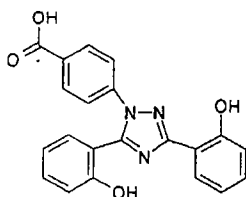
DRUG CODE: ICL670

CAS#: 201530-41-8

DIVISION: HFD-180

DRUG NAME: Exjade[®]/Deferasirox

CHEMICAL STRUCTURE:



SPONSOR: Novartis Pharmaceuticals Corporation, East Hanover, NJ

LABORATORY: Novartis Pharmaceuticals Corp., East Hanover, NJ

CARCINOGENICITY STUDY REPORT DATE: September 27, 2004

THERAPEUTIC CATEGORY: ICL-670 is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis).

PHARMACOLOGICAL CLASSIFICATION: Iron chelator

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): ICL670 was negative in the Ames test and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests.

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RAT CARCINOGENICITY STUDY :

RAT STUDY DURATION (weeks): 104-Week

STUDY STARTING DATE: May 21, 2001

STUDY ENDING DATE: September 27, 2004

RAT STRAIN: IGS Wistar Hannover Rat; WI (— BRL/ Han) IGS BR

ROUTE: Oral (gavage)

DOSING COMMENTS: The dose selection for the current study was based upon maximum tolerated dose (MTD, toxicity endpoint) determined in a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. Based on the results of this study, the doses for the carcinogenicity study were selected as 0 (control feed, Group 1), 0 (iron supplemented feed, Group 2), 25 (iron-supplemented feed, Group 3), 50 (iron-supplemented feed, Group 4) and 100 (iron-supplemented feed, Group 5) mg/kg/day. Beginning pretest Day 8 (May 14, 2001), Group 1 controls received standard certified rodent diet and animals in Groups 2-5 received certified rodent diet supplemented with iron (approximately 1680 ppm $\pm$ 10%), *ad libitum*. These dose levels were subsequently decreased to 15, 30 and 60 mg/kg/day beginning on Day 29, based upon the recommendations of CDER Executive CAC (ExecCAC Meeting Minutes dated June 5, 2001, Appendix-1).

NUMBER OF RATS:

- Control-1 (C1, standard diet): 50/sex
- Control-2 (C2, iron-supplemented diet): 50/sex
- Low Dose (LD, iron-supplemented diet): 50/sex
- Middle Dose (MD, iron-supplemented diet): 50/sex
- High Dose (HD, iron-supplemented diet): 50/sex

RAT DOSE LEVELS (mg/kg/day):

- Low Dose: 25→15*
- Middle Dose: 50→30*
- High Dose: 100→60*

*Doses were decreased on Day 29 of the study as per ExecCAC recommendations (June 5, 2001)

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible):

MTD. Doses were selected based upon the toxicity endpoint from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. High dose of 180 mg/kg/day was lethal and exceeded maximum tolerated dose (MTD). ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity

(cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, MTD was identified between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to MTD in females. Based on the results of this study, the doses for the carcinogenicity study were selected as 0 (control feed), 0 (iron supplemented feed), 25, 50 and 100 mg/kg/day. These dose levels were subsequently decreased to 15, 30 and 60 mg/kg/day beginning on Day 29, based upon the recommendations of CDER Executive CAC (June 5, 2001, Appendix-1).

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Yes. The sponsor submitted a dose selection and protocol under Amendment 015 dated April 30, 2001. The Exec CAC dated June 5, 2001 (Appendix-1) did not concur with the sponsor's proposed doses of 0, 25, 50 and 100 mg/kg/day. The Committee recommended doses of 15, 30 and 60 mg/kg/day due to concerns for toxicity at higher doses and a concern that the proposed high dose of 100 mg/kg/day is above the MTD.

RAT CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Negative.

RAT TUMOR FINDINGS: In this study, one-sided trend test was performed for comparisons across Groups 2, 3, 4 and 5 using Peto's survival adjusted trend test. For males and females, the one-sided overall trend tests were not found to be significant in any case ($p > 0.05$). In addition to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The increased incidence of tumors in the treated groups 3, 4 and 5 was not considered significant for all neoplastic lesions, as their p-values were > 0.05 . The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. However, when comparing the tumor incidence in Group 1 to Group 2, the differences were found to be significant for combined c-cell adenomas and carcinomas in the thyroid of males if this tumor is classified as a rare tumor. Overall, there was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

RAT STUDY COMMENTS: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). The doses were selected based on the maximum tolerated dose (MTD) estimation from the results of a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. The sponsor first initiated the study at their proposed doses of 25, 50 and 100 mg/kg/day and these dose levels were subsequently decreased to 15, 30 and 60 mg/kg/day on Day 29 based upon the recommendations (15, 30 and 60 mg/kg/day) of CDER Executive CAC (June 5, 2001, Appendix-1). The sponsor submitted limited historical control data from this testing facility from three studies conducted during the period of 1999-2003. Overall, the conduct of the study was found to be acceptable.

COVER SHEET FOR CARCINOGENICITY STUDY IN THE RAT

1. **Study Number:** 017022
2. **Name of Laboratory:** Novartis Pharmaceuticals, East Hanover, NJ
3. **Strain:** IGS Wistar Hannover Rat; WI C¹ BRL/ Han) IGS BR
4. **No./Sex/Group:** 50
5. **Doses (0, L, M, and H):** 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). Beginning pretest Day 8 (May 14, 2001), Group 1 controls received standard certified rodent diet and animals in Groups 2-5 received certified rodent diet supplemented with iron (approximately 1680 ppm ± 10%), *ad libitum*.
6. **Basis for Dose Selection Stated:** Yes. Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. High dose of 180 mg/kg/day was lethal and exceeded maximum tolerated dose (MTD). ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, MTD was identified between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to MTD in females. Based on the results of this study, the sponsor initiated the carcinogenicity study at 0 (control feed), 0 (iron supplemented feed), 25, 50 and 100 mg/kg/day. The Exec CAC dated June 5, 2001 (Appendix-1) did not concur with the sponsor's proposed doses of 0, 25, 50 and 100 mg/kg/day. The Committee recommended doses of 15, 30 and 60 mg/kg/day due to concerns for toxicity at higher doses and a concern that the proposed high dose of 100 mg/kg/day is above the MTD. Based upon the recommendations of CDER Executive CAC (June 5, 2001, Appendix-1), these dose levels were subsequently reduced to 15, 30 and 60 mg/kg/day beginning on Day 29.
7. **Interim Sacrifice:** None
8. **Total Duration (Weeks):** 104 Weeks
9. **Week/Site for First Tumor:** The sponsor did not provide chronological tumor data.
10. **Number Alive at Termination:** The survival data is shown in the following table (from page 27 of the study report).

Table 4-1 Summary of survival ratios with % survival

Group:	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Dose:	0	0	25→15	50→30	100→60	0	0	25→15	50→30	100→60
Week										
14	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	49 (98%)	50 (100%)	50 (100%)
26	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	48 (96%)	49 (98%)	50 (100%)
54	50 (100%)	50 (100%)	49 (98%)	49 (98%)	49 (98%)	48 (96%)	50 (100%)	48 (96%)	47 (94%)	49 (98%)
78	47 (94%)	45 (90%)	48 (96%)	46 (92%)	45 (90%)	40 (80%)	46 (92%)	45 (90%)	41 (82%)	43 (86%)
Term	42 (84%)	40 (80%)	38 (76%)	36 (72%)	40 (80%)	31 (62%)	32 (64%)	34 (68%)	29 (58%)	34 (68%)

Dose = mg/kg/day.

The dose decrease for each ICL670 treated group began on day 29.

11. Statistical Methods Used:

Mortality data: The survival data was analyzed by using Kaplan-Meier product limit method using SAS/STAT module. The significance of group effects on mortality rates was assessed by applying the logrank test. No Tarone's trend test and no pairwise comparison were conducted, as the group effect was not found to be significant for both sexes.

Tumor data: The trend test was performed for Groups 2, 3, 4 and 5 using Peto's survival adjusted one-sided trend test. According to the recommendations of Lin and Rahman (Lin KK and Rahman MA. 1998. *J Biopharm Stat* 8: 1-15), the dose-related increase in tumor incidence was considered significant if the p-value is ≤ 0.025 for rare tumors and ≤ 0.005 for common tumors (historical incidence of more than 1%). In addition to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. As per the recommendations of Lin and Rahman (1998), the increased tumor rate in a treated group compared to control was considered significant when the p-value is ≤ 0.05 for a rare tumor, or ≤ 0.01 for a common tumor.

12. Attach Tumor and Non-Tumor Data for Each Tissue: List of neoplastic and non-neoplastic lesions is attached in Appendix-2.

CARCINOGENICITY:

Study Title: 2-Year Oral (Gavage) Carcinogenicity Study with ICL670 in Wistar Hannover Rats

Key Study Findings: In this study, no test article-related mortality or moribundity was evident upon histopathological assessment. At study termination, 72-84% survival was observed in the males and 58-68% survival was observed in the females. There were no statistical differences in survival between the control groups and ICL670-treated dose groups. There were no test article-related effects noted during palpable mass examination. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates. Based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks did not appear to be carcinogenic at doses up to 100→60 mg/kg/day.

Study Number: 017022

Volume #, and Page #: Page 1 of Report 017022

Conducting Laboratory and Location: Novartis Pharmaceuticals Inc., East Hanover, NJ

Date of Study Initiation: May 21, 2001

Date of Study Completion: September 27, 2004

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, []

CAC Concurrence: Yes. Exec CAC dated June 5, 2001 (Appendix-1)

Study Type: 2-Year bioassay

Species/Strain: Rats/Wistar Hannover

Number/Sex/Group; Age at Start of Study: 50/sex/group; Age: 8 weeks

Body Weight: Males: 204.5 to 316.8 g; Females: 148.3 to 209.1 g

Animal Housing: Animals were housed in pairs in stainless steel cage.

Formulation/Vehicle: Suspension in 0.5% (w/v) Klucel (type HF), NF

Drug Stability/Homogeneity: ICL670 suspensions in 0.5% Klucel, 0.75 mg/mL to 100 mg/mL, were found to be stable for at least [] stored at 6°C and for at least [] stirring at room temperature. The 0.5% Klucel was found to be suitable for use for at least [] stirring at room temperature and at least [] stored at 6°C. The uniformity of the dosing formulations was confirmed. Samples prepared for Weeks 1, 5, 9, 24, 39, 54, 69, 84, and 104 were tested and the concentrations were [] of target concentrations. Nine control samples were tested, and no ICL670 was detected.

Methods: The purpose of this study was to determine the potential carcinogenicity profile of ICL670, when administered orally to rats fed an iron supplemented diet for at least 104 weeks. ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented

with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). Beginning pretest Day 8 (May 14, 2001), Group 1 controls received standard certified rodent diet and animals in Groups 2-5 received certified rodent diet supplemented with iron (approximately 1680 ppm ± 10%), *ad libitum*. ICL670 doses were decreased on Day 29 of the study based upon the recommendations of CDER ExecCAC (ExecCAC meeting minutes dated June 5, 2001, Appendix-1). The dosing volume for all dose groups was 5 mL/kg.

Doses: 0 (standard feed, Group 1), 0 (feed supplemented with iron, Group 2), 25→15 (feed supplemented with iron, Group 3), 50→30 (feed supplemented with iron, Group 4) and 100→60 mg/kg/day (feed supplemented with iron, Group 5)

The following table presents the study design (from page 19 of the study report)

3.3.3 Study design

Pretest period: Approximately 2 weeks

Duration of dosing: At least 104 weeks

Table 3-1 Study design, animal allocation and test article doses

Group	Number/sex	Animal Numbers		Dose (mg/kg/day)	Concentration (mg/mL)
		males	females		
1 Control (standard feed)	50	1001-50	1501-50	0	0
2 Control (iron supplemented feed)	50	2001-50	2501-50	0	0
3 Low (iron supplemented feed)	50	3001-50	3501-50	25→15*	5→3
4 Mid (iron supplemented feed)	50	4001-50	4501-50	50→30*	10→6
5 High (iron supplemented feed)	50	5001-50	5501-50	100→60*	20→12

*Dose decrease effective day 29.

Animals were fasted daily for approximately 4 hours, whenever possible, prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing, whenever possible. All changes to this intended schedule were acknowledged/approved and documented in the raw data file. Feeding/fasting times were documented in the raw data. All animals were acclimated to fasting procedures for 5 days prior to the start of dosing (i.e., for an approximate 6 hour daily fast).

Dosing was not performed on 07-Feb-2003 (day 628), 17-Feb-2003 (day 638) and 18-Feb-2003 (day 639) due to inclement weather and/or site closure. These occurrences had no impact on the study.

Basis of dose selection: Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. High dose of 180 mg/kg/day was lethal and

exceeded maximum tolerated dose (MTD). ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, MTD was identified between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to MTD in females. Based on the results of this study, the sponsor initiated the carcinogenicity study at 0 (control feed), 0 (iron supplemented feed), 25, 50 and 100 mg/kg/day. The Exec CAC dated June 5, 2001 (Appendix-1) did not concur with the sponsor's proposed doses of 0, 25, 50 and 100 mg/kg/day. The Committee recommended doses of 15, 30 and 60 mg/kg/day due to concerns for toxicity at higher doses and a concern that the proposed high dose of 100 mg/kg/day is above the MTD. These dose levels were subsequently decreased to 15, 30 and 60 mg/kg/day beginning on Day 29, based upon the recommendations of CDER Executive CAC (June 5, 2001, Appendix-1).

Route of administration: Oral (gavage)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Satellite PK: No. Plasma samples were analyzed for toxicokinetic assessment during Weeks 4, 8 (following the dose decrease) and 52. Two animals/sex/group/time point were bled at approximately 0.5, 2, 4, and 7 and 24 hours post dose.

Deviations from original study protocol: During full feeder weight collection on June 17, 2002 (Day 393), full feeder weights for animal nos. 3501- 3526 were inadvertently mis-weighed, as the feed receptacle was inadvertently not tared to zero prior to weighing. As a result of this error, empty feeder weights were not weighed for these animals on June 24, 2002 (Day 400) and food consumption was not calculated. Instead, an entry "NW" (not weighed) was made for these animals. This protocol deviation does not appear to have any significant impact on the study outcome.

Statistical methods:

Mortality data: The survival data was analyzed by using Kaplan-Meier product limit method using SAS/STAT module. The significance of group effects on mortality rates was assessed by applying the logrank test. No Tarone's trend test and no pairwise comparison were conducted, as the group effect was not found to be significant for both sexes.

Tumor data: The trend test was performed for Groups 2, 3, 4 and 5 using Peto's survival adjusted one-sided trend test. According to the recommendations of Lin and Rahman (Lin KK and Rahman MA. 1998. *J Biopharm Stat* 8: 1-15), the dose-related increase in tumor incidence was considered significant if the p-value is ≤ 0.025 for rare tumors and ≤ 0.005 for common tumors (historical incidence of more than 1%). In addition to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. As per the recommendations of Lin and Rahman (1998), the increased tumor rate in a treated group compared to control was considered significant when the p-value is ≤ 0.05 for a rare tumor, or ≤ 0.01 for a common tumor.

Observations and Times:

Clinical Signs: Clinical signs were observed once daily.

Body Weights: Body weights were recorded once during pretest, and once weekly during Weeks 1 to 14; every four weeks from Weeks 18 to 78; and every 2 weeks thereafter.

Food Consumption: Food consumption was recorded once weekly during Weeks 1 to 14, every fourth week from Weeks 18 to 78 and every 2 weeks thereafter during the dosing period.

Ophthalmoscopy: Ophthalmoscopic examinations (using a Fison Indirect Ophthalmoscope) were conducted on all animals during pretest and on all surviving animals during Weeks 12, 25, 51, 65 and 78. During Weeks 91 and 104 only surviving control and high-dose rats were examined.

Palpable Mass Examination: Palpable mass examinations were conducted monthly for the first 12 months of the study and every two weeks thereafter.

Clinical Pathology: Clinical pathology assessments (hematology and serum chemistry) were conducted on the last 10 surviving animals/sex/group during Weeks 13, 26 and 52.

Plasma and Tissue Iron Analysis: Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) were determined in the serum. In addition, liver (section of right lobe) and kidney (right pole) were collected after exsanguinations from the last 10 surviving rats/sex/group at the terminal sacrifice for the determination of iron content by means of AAS following mineralization of the tissue samples.

Histopathology: The tissues specified in the following list (from page 24 of the study report) were examined for histopathology:

Tissue list for collection and/or processing (P)

P	adrenal	P	ovary
P	aorta	P	pancreas
	blood and bone marrow smears	P	parathyroid
P	bone marrow (in bone)	P	pituitary
P	brain	P	preputial gland
P	cecum	P	prostate
P	cervix	P	rectum
P	clitoral gland	P	salivary gland
P	colon	P	sciatic nerve
P	duodenum	P	seminal vesicle
P	epididymis	P	skeletal muscle
P	esophagus	P	skin
P	eye	P	spinal cord
P	femur/tibia	P	spleen
P	Harderian gland	P	sternum
P	heart	P	stomach
P	ileum	P	testis
P	jejunum	P	thymus
P	kidney	P	thyroid
P	lacrimal gland	P	tongue
P	liver	P	trachea
P	lung	P	urinary bladder
P	lymph node – bronchial	P	uterus
P	lymph node – mandibular	P	vagina
P	lymph node – mesenteric	P	macroscopic lesions
P	mammary gland		animal identification
	nasal passage		

Toxicokinetics: Blood was collected from the first 10 surviving animals/sex in each group during Weeks 4, 8 (4 weeks after the dose decrease) and 52. Two surviving animals/sex/group were bled at approximately 0.5, 2, 4, 7 and 24 hours post dose.

Results:

Mortality: No test article-related mortality was observed during the study. There were no apparent differences in mortality rate between the control groups or between the Group 2

control (iron supplemented controls) and test article-treated dose groups. The survival ratios are presented in the following table (from page 27 of the study report).

Table 4-1 Summary of survival ratios with % survival

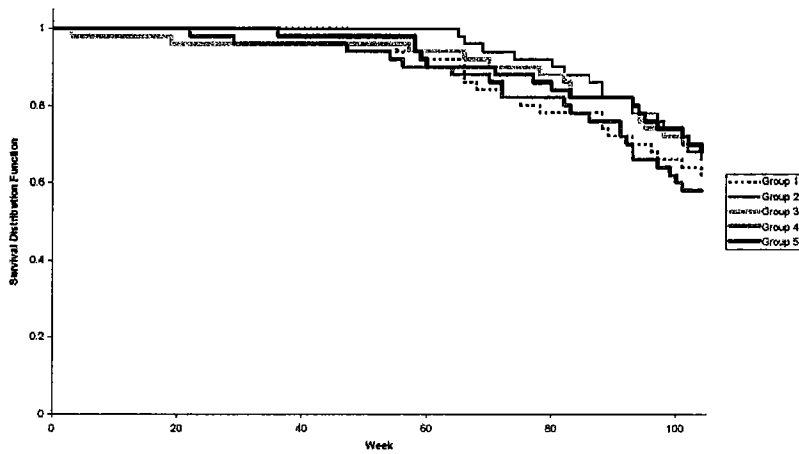
Group:	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Dose:	0	0	25→15	50→30	100→60	0	0	25→15	50→30	100→60
Week										
14	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	49 (98%)	50 (100%)	50 (100%)
26	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	48 (96%)	49 (98%)	50 (100%)
54	50 (100%)	50 (100%)	49 (98%)	49 (98%)	49 (98%)	48 (96%)	50 (100%)	48 (96%)	47 (94%)	49 (98%)
78	47 (94%)	45 (90%)	48 (96%)	46 (92%)	45 (90%)	40 (80%)	46 (92%)	45 (90%)	41 (82%)	43 (86%)
Term	42 (84%)	40 (80%)	38 (76%)	36 (72%)	40 (80%)	31 (62%)	32 (64%)	34 (68%)	29 (58%)	34 (68%)

Dose = mg/kg/day.

The dose decrease for each ICL670 treated group began on day 29.

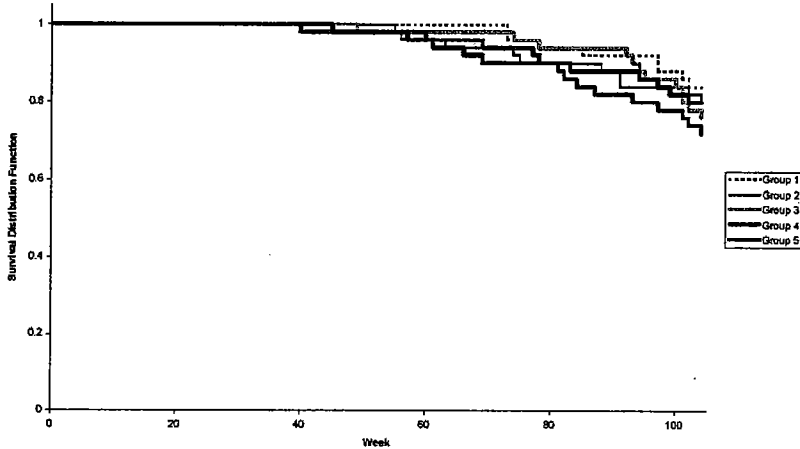
Survival curves are shown in the following figures (from page 46 of the study report).

Figure 2-1 Survival curves for each group of females based on Kaplan-Meier estimates



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Figure 2-2 Survival curves for each group of males based on Kaplan-Meier estimates



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Clinical Signs: Treatment-related clinical signs included slight increases in the incidence of perineal staining (wet staining in the males and wet or dry staining in the females) in both sexes at 100→60 mg/kg/day. In addition, excessive nail growth was observed in all dose groups but was more evident in the iron supplemented controls and test article-treated dose groups. Atypical hairloss (generalized hairloss which progressed to the whole body) and reddened skin was also observed in individual iron supplemented and ICL670-treated animals during the study. The affected animals typically displayed a combination of these signs or all of these clinical signs.

Body Weights: The mean initial and final body weight of control (Control 1) males was 258.4 and 571.9 g, respectively. The mean initial and final body weight of control (Control 1) females was 180.8 and 350.7 g, respectively. The mean initial and final body weight of control (Control 2) males was 256.0 and 549.4 g, respectively. The mean initial and final body weight of control (Control 2) females was 181.8 and 365.9 g, respectively. Statistically significant decreases in mean body weight were observed in the males at high-dose (approximate decreases of 5-6% compared to Group 2 controls) on Days 176-372 and Day 484. Thereafter, the decreases were less than 5% and were negligible by the end of the study. Statistically significant decreases in mean absolute body weight gain were also observed in the males at 100→60 mg/kg/day on the following days: Days 22-64, 85, 148-400, 456-512 and Day 568 (approximate decreases of 9-14% compared to Group 2 controls). The following table shows the body weight data (g).

Day	Males					Females				
	+Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
1	258.4	256.0	257.2	259.8	254.6	180.8	181.8	182.6	179.9	179.7
15	304.5	299.7	303.1	305.9	294.9	201.5	201.6	201.8	198.8	197.1
29	336.4	329.9	331.2	335.8	318.8	211.6	210.2	211.0	207.5	206.9
92	403.6	391.5	395.2	400.9	379.0	234.8	235.9	236.0	229.1	231.2
176	444.8	431.8	438.5	441.7	411.4*	250.7	253.7	252.3	247.8	247.8
288	488.9	471.3	477.0	484.2	446.1*	270.5	275.9	277.8	271.3	271.6
372	516.1	493.7	502.8	511.9	466.6*	289.0	293.6	293.9	297.9	291.2
540	564.9	536.4	549.5	557.5	509.6	331.2	339.5	328.7	338.6	336.9
722	571.9	549.4	571.8	572.0	539.0	350.7	365.9	356.3	358.6	366.1
Body Weight (% of Control 2)										
722		100%	104%	104%	98.1%		100%	97.4%	98.0%	100%

*: Significantly different from Control (2), $p = 0.05$ with Dunnett's test

+:

Group 1: Control 1 (Standard feed)

Group 2: Control 2 (iron supplemented feed)

Group 3: 25→15 mg/kg/day (iron supplemented feed)

Group 4: 50→30 mg/kg/day (iron supplemented feed)

Group 5: 100→60 mg/kg/day (iron supplemented feed)

Food Consumption: The mean initial and final food consumption in Control 2 males was 22.0 and 21.3 g/animal/day, respectively. The mean initial and final food consumption in Control 2 females was 17.94 and 16.8 g/animal/day, respectively. Food consumption was analyzed comparing Group 2 controls (iron supplemented) to the ICL670-treated dose groups. Slight treatment-related decreases in mean food consumption values were observed in males at 100→60 mg/kg/day. Statistically significant decreases were also observed in males at high-dose on the following days: Day 36-50, 148, 456, 484, 540 and 554. There were no test treatment-related effects on mean food consumption in the females. The following table shows the food consumption (g/animal/day) data.

Day of Study	Males			Females		
	Low-Dose	Mid-Dose	High-Dose	Low-Dose	Mid-Dose	High-Dose
9	21.8	21.7	21.0	18.9	18.0	18.7
15	24.0	23.4	22.9	18.7	18.9	18.6
29	23.2	23.5	22.2	17.9	17.2	17.8
43	22.9	22.8	22.0	17.5	16.8	17.4
57	22.4	22.8	21.9	17.3	17.1	16.9
78	22.7	22.2	22.0	18.0	17.4	17.7
92	21.2	21.5	21.3	17.0	17.0	16.9

Ophthalmology: Ophthalmoscopic examinations performed during the course of the study did not reveal any ocular changes attributable to treatment with ICL670.

Palpable Mass: There was no apparent increase in the incidence of palpable masses observed during the course of the study or prior to scheduled necropsy. A summary of the palpable mass incidence at the last scheduled necropsy is presented in the following table (page 29 of the study report).

Table 4-2 Palpable mass incidence at final palpable mass exam (day 723)

Group Dose (mg/kg/day)	Incidence (% incidence)	
	Males	Females
1 0 (standard diet)	9/42 (21.4%)	9/32 (28.1%)
2 0 (iron supplemented diet)	6/41 (14.6%)	8/34 (23.5%)
3 25→15 mg/kg/day (iron supplemented diet)	3/39 (7.7%)	9/35 (25.7%)
4 50→30 mg/kg/day (iron supplemented diet)	5/36 (13.9%)	2/29 (6.9%)
5 100→60 mg/kg/day (iron supplemented diet)	6/40 (15%)	5/34 (14.7%)

Hematology: No treatment-related changes were observed.

Serum Iron Analysis: Serum iron analyses revealed slight increases in serum iron concentrations in the iron supplemented controls (both sexes at Week 13), in males at 100→60 mg/kg/day (Weeks 13, 26 and 52) and in the females at all dose levels (Weeks 13, 26 and 52). Unsaturated iron binding capacity (UIBC) was decreased minimally in iron-supplemented control rats (both sexes), in Group 5 males and Groups 3, 4, and 5 females at all time points, and increased marginally in Group 3 males at all time points. However, the total iron binding capacity (TIBC) was not altered.

Tissue Iron Analysis: Dose-related decreases in mean liver and kidney iron content were evident in males at all doses when compared to the iron supplemented controls (approximately 57-73% reduction in the liver and approximately 40-70% reduction in the kidney) and when compared to control males fed the standard rodent diet (approximately 40-63% reduction in the liver and 24-61% reduction in the kidney). In addition, dose-related decreases in mean liver and kidney iron content were also observed in females at all doses when compared to the iron supplemented controls (approximately 34-72% reduction in the liver and approximately 38-74% reduction in the kidney) and when compared to control females fed the standard rodent diet (approximately 28-69% reduction in the liver and approximately 18- 66% reduction in the kidney). Iron supplementation of the controls increased the tissue iron content by 28-41% in males and by 9-32% in the females.

Macroscopic Examinations: There were no treatment-related macroscopic findings.

Histopathology:

Non-Neoplastic: There appears to be no significant treatment-related findings. However, histopathological changes were seen in different tissues, which do not appear to be treatment-related either in the absence of a dose-response or presence of these changes in the control animals also. The following table shows some of the histopathological observations.

Tissue Findings	Males					Females				
	Dose (mg/kg/day)					Dose (mg/kg/day)				
	0 (n*=50)	0 (n=50)	25→15 (n=50)	50→30 (n=50)	100→60 (n=50)	0 (n*=50)	0 (n=50)	25→15 (n=50)	50→30 (n=50)	100→60 (n=50)
HEART										
Inflammation, acute								1		
Fibrosis, pericardium									1	
Periarteritis		1		1						
Mineralization, aorta	1								1	
Hyperplasia, endocardium										1
SALIVARY GLAND										
Inflammation	1		1		2		1	1		
Metaplasia, squamous			1							
Atrophy, acinal, focal								1		1
Hemorrhage									1	
STOMACH										
Edema, non-glandular		1	1	1		1	4	2	2	
Erosion, non-glandular		1		2	1		1	4		1
Hyperplasia, mucosa, glandular, focal						1				1
Inflammation, nonglandular		1	1	1		1	2	1	1	
Ulcer, nonglandular				1			2	2	2	
LIVER										
Hyperplasia, bile duct	7	5	10	8	7	15	11	10	11	11
Inflammation	5	3	8	8	7	3	3	5	8	4
Necrosis, focal	1	1	1					1	1	

Necrosis, centrilobular			1							1
Extramedullary hematopoiesis	2	3	2	3	2	3	3	3	2	2
Necrosis								1		
Fibrosis, periportal									1	
ADRENAL										
Atrophy, cortex					1			2		
Hypertrophy, cortex, focal			1	2		1		3	1	1
Infiltrate, lymphocyte					1					
Necrosis, cortex							1	1	2	2
PITUITARY										
Hemorrhage				1	1					
THYROID										
Inflammation								1	1	1
KIDNEY										
Hyperplasia, epithelium, pelvis					1					
Inflammation	1	1	5	3	4	1		3	4	2
Necrosis, papillary	1				1			1		1
Necrosis, tubular			1	2	2	4	4	4	4	4
Pyelonephritis	1							1	2	1
SEMINAL VESICLE										
Inflammation	1		2	1	3					
TESTIS										
Atrophy, somniferous tubule	7	9	9	10	8					
URINARY BLADDER										
Hyperplasia, transitional	1				2			1	1	1
THYMUS										
Hyperplasia, focal		1	1	2		3		2		1
SPLEEN										
Necrosis, focal										1
Hyperplasia, lymphoid										2

*: n = Number of animals examined

Neoplastic: In this study, one-sided trend test was performed for comparisons across Groups 2, 3, 4 and 5 using Peto's survival adjusted trend test. For males and females, the one-sided overall trend tests were not found to be significant in any case ($p > 0.05$). In addition

to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The increased incidence of tumors in the treated groups 3, 4 and 5 was not considered significant for all neoplastic lesions, as their p-values were > 0.05. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. However, when comparing the tumor incidence in Group 1 to Group 2, the differences were found to be significant ($p = 0.0344$) for combined c-cell adenomas or carcinomas in the thyroid of males if this tumor is classified as a rare tumor. Overall, there was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates. It is to be mentioned here that the sponsor provided limited historical control data for this testing facility from three different studies conducted during the period of 1999-2003. The tumor incidences are shown in the following table (data extracted from page 1003-1035 of sponsor's report).

Organ/Tumor	Control 1 (n = 50)	Control 2 (n = 50)	25→15 mg/kg/day (n = 50)	50→30 mg/kg/day (n = 50)	100→60 mg/kg/day (n = 50)	p-Value*	Historical Control Incidences
FEMALES							
Adrenals							
Cortical adenoma	2 (0%)	1 (2%)	0 (0%)	1 (2%)	1 (2%)	0.4446	1.76%
Pheochromocytoma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.62%
Brain							
Granular cell tumor	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2595	0.6%
Clitoral Gland							
Squamous cell carcinoma	0 (0%)	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/48 (2.1%)	0.2558	NA
Liver							
Hepatocellular carcinoma	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0.9380	0.6%
Lung							
Adenoma, bronchioloalveolar	0 (0%)	1 (2%)	1 (2%)	0/49 (0%)	1/49 (2%)	0.5378	NA
Mammary Gland							
Adenocarcinoma	6 (12%)	3 (6%)	1 (2%)	2 (4%)	0 (0%)	0.9313	12.3%
Adenolipoma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Adenoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2457	2.9%
Fibroadenoma	4 (8%)	9 (18%)	8 (16%)	3 (6%)	7 (14%)	0.7502	18.2%
Fibroma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Muscle							
Rhabdomyosarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7403	NA
Ovary							
Adenocarcinoma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Luteal cell tumor	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2593	NA
Thecal cell tumor	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	1.2%
Pancreas							
Islet cell adenoma or carcinoma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.0%
Parathyroid							

Adenoma	0/46 (0%)	1/47 (2.1%)	2/47 (4.2%)	0/47 (0%)	0/48 (0%)	0.9390	NA
Pituitary							
Adenoma or carcinoma, pars distalis	28 (56%)	29 (58%)	24 (48%)	28 (56%)	28 (56%)	0.4263	NA
Adenoma, pars intermedia	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Carcinoma, pars intermedia	0 (0%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	0.7989	NA
Reproductive Tract							
Polyp, Endometrial stromal polyp or sarcoma	4 (8%)	8 (16%)	8 (16%) ¹	7 (14%)	3 (6%)	0.9366	NA
Skin							
Basal cell tumor	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.0%
Fibroma or fibrosarcoma	1 (2%)	0 (0%)	1 (2%)	0 (0%)	2 (4%)	0.1091	NA
Fibrosarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	1 (2%)	0.3126	NA
Stomach							
Sarcoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2585	NA
Squamous cell carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2593	NA
Systemic							
Histiocytic sarcoma	0 (0%)	0 (0%)	3 (6%)	0 (0%)	0 (0%)	0.8352	NA
Thymus							
Thymoma	2 (4%)	5 (10%)	5 (10%)	6 (12%)	7 (14%)	0.2550	11.8%
Thyroid							
C-cell adenoma or carcinoma	5/49 (10.2%)	13 (26%)	7 (14%)	7 (14%)	5 (10%)	0.9684	NA
Follicular adenoma or adenocarcinoma	0/49 (0%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)	0.8314	NA
Uterus							
Adenocarcinoma	0 (0%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)	0.5636	3.5%
Choriocarcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2500	NA
Endometrial stromal polyp or sarcoma	4 (8%)	6 (12%)	7 (14%)	7 (14%)	3 (6%)	0.8408	1.76%
Vagina							
Granular cell tumor	2 (4%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	1.0000	10.6%
Polyp	0 (0%)	2 (4%)	1 (2%)	0 (0%)	0 (0%)	0.9743	NA
Whole Body							
Hemangiosarcoma	1 (2%)	2 (4%)	1 (2%)	0 (0%)	0 (0%)	0.9851	NA
Leiomyosarcoma	3 (6%)	2 (4%)	0 (0%)	1 (2%)	1 (2%)	0.7016	NA
Lipoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7481	NA
Nerve sheath tumor	1 (2%)	0 (0%)	1 (2%)	0 (0%)	2 (4%)	0.1144	NA
MALE							
Adrenal							
Cortical adenoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (4%)	0.0635	1.18%
Pheochromocytoma	3 (6%)	2 (4%)	1 (2%)	1 (2%)	0 (0%)	0.9380	2.9%

Brain							
Astrocytoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2597	1.2%
Granular cell tumor	0 (0%)	0 (0%)	2 (4%)	0 (0%)	1 (2%)	0.5000	1.17%
Epididymides							
Mesothelioma	0 (0%)	0 (0%)	2 (4%)	2 (4%)	0 (0%)	0.6807	NA
Kidney							
Adenoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2597	NA
Transitional cell carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4913	NA
Liver							
Hepatocellular adenoma or carcinoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)	2 (4%)	0.2531	NA
Hepatocellular carcinoma	0 (0%)	1 (2%)	0 (0%)	2 (4%)	2 (4%)	0.1795	1.8%
Lung							
Adenoma, bronchioloalveolar	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2105	NA
Muscle							
Fibrosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4944	NA
Pancreas							
Islet cell adenoma or carcinoma	2 (4%)	0 (0%)	3 (6%)	3 (6%)	2 (4%)	0.2749	NA
Parathyroid							
Adenoma	2/49 (4.1%)	1 (2%)	1/48 (2.1%)	1/49 (2.0%)	0/48 (0%)	0.8312	1.17%
Pituitary							
Adenoma or carcinoma, pars distalis	17 (34%)	15/49 (30.6%)	19 (38%)	15 (30%)	21 (42%)	0.1706	NA
Adenoma, pars intermedia	1 (0%)	1/49 (2.0%)	2 (4%)	0 (0%)	0 (0%)	0.9278	NA
Carcinoma, pars distalis	0 (0%)	0/49 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2575	0.6%
Prostate							
Adenocarcinoma	0 (0%)	1 (2%)	0 (0%)	1 (2%)	0 (0%)	0.8050	NA
Rectum							
Sarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7403	NA
Salivary Gland							
Adenocarcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4935	NA
Skin							
Fibroma or fibrosarcoma	1 (2%)	2 (4%)	0 (0%)	0 (0%)	2 (4%)	0.4172	NA
Testis							
Interstitial cell tumor	2 (4%)	3 (6%)	1 (2%)	1 (2%)	0 (0%)	0.9787	1.76%
Thymus							
Thymoma	2/49 (4.1%)	2 (4%)	0/49 (0%)	1/48 (2.1%)	3 (6%)	0.2082	3.5%
Thyroid							

C-cell adenoma or carcinoma	3 (6%)	10 (20%)	7 (14%)	10 (20%)	9 (18%)	0.4804	NA
Whole Body							
Hemangiosarcoma	0 (0%)	1 (2%)	1 (2%)	5 (10%)	0 (0%)	0.6704	NA
Leiomyosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4935	NA
Lipoma	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA

*: p-values are from one-sided trend tests across Groups 2 to 5.

Trend Test: $p \leq 0.025$ (Significant for rare tumors); $p \leq 0.005$ (Significant for common tumors)

Pairwise Comparison: $p \leq 0.05$ (Significant for rare tumors); $p \leq 0.01$ (Significant for common tumors)

Toxicokinetics: Initially, at Week 4, the exposure [$AUC_{(0-24h)}$] appeared to increase over-proportionally with the dose in both sexes, between doses of 25 to 100 mg/kg/day. There was an apparent gender difference in exposure at the beginning of the study. Exposure in females was found to be higher at the mid- and high-dose when compared to male rats. The exposure increased approximately two-fold between Week 8 and 52 at the same dose, based on $AUC_{(0-24h)}/\text{dose}$. After 8 and 52 weeks of treatment, the exposure increased over-proportionally between doses of 15 to 60 mg/kg/day, in both sexes. After 52 weeks of treatment, there was no apparent gender differences in exposure to ICL670 at the low- and mid-dose (15 and 30 mg/kg/day); however, the exposure was found to be higher in males than in females at the high dose (60 mg/kg/day). The mean TK parameters are shown in the following table (from page 31 of the study report).

Table 4-3 Mean toxicokinetic parameters of total ICL670 in rats

Doses (mg/kg/day)	Sampling period (week)	C_{max} ($\mu\text{mol/L}$)		$AUC_{(0-24h)}$ ($\mu\text{mol.h/L}$)		$AUC_{(0-24h)}/\text{dose}$ ($\mu\text{mol.h/L}/(\text{mg/kg/day})$)	
		male	female	male	female	male	female
25	4	23.7	18.3	71.0	30.7	2.84	1.23
15*	8	5.13	13.6	10.9	20.4	0.724	1.36
15*	52	12.9	13.2	32.8	42.7	2.19	2.85
50	4	31.7	44.5	89.9	165	1.80	3.30
30*	8	12.9	35.5	43.3	72.2	1.44	2.41
30*	52	41.6	46.2	123	135	4.11	4.52
100	4	189	310	607	921	6.07	9.21
60*	8	81.5	61.7	217	115	3.61	1.91
60*	52	178	76.9	543	387	9.05	6.45

*Doses were reduced on day 29.

Summary Study Findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The study was considered adequate. The test model (Wistar Hannover rats) was also considered appropriate. Doses [0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/ kg/ day (feed supplemented with iron)] were used as per ExecCAC recommendations. Doses were reduced on Day 29 as per the recommendations of the Executive CAC. Overall, the conduct of the study was considered appropriate and acceptable. There was adequate number of animals at the end of the study in each group for statistical considerations. It is to be mentioned here that the sponsor submitted limited historical control data from this testing facility from three studies conducted during the period of 1999-2003.

Evaluation of tumor findings: In this study, the trend test was performed for Groups 2, 3, 4 and 5 using Peto's survival adjusted trend test. For males and females, the one-sided overall trend tests were not found to be significant in any case ($p > 0.05$). According to the recommendations of Lin and Rahman (Lin KK and Rahman MA. 1998. *J Biopharm Stat* 8: 1-15), the dose-related increase in tumor incidence was considered significant if the p-value is ≤ 0.025 for rare tumors and ≤ 0.005 for common tumors (historical incidence of more than 1%). Using these criteria, the trend test was found to be non-significant, as all the p-values were > 0.025 . In addition to the overall trend test, pairwise comparisons were also made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. For each pair-wise comparison, only the two considered groups were used in the analysis. In both sexes, there was no tumor or tumor combinations for which p-value was found to be ≤ 0.05 . As per the recommendations of Lin and Rahman (1998), the increased tumor rate in a treated group compared to control was considered significant when the p-value is ≤ 0.05 for a rare tumor, or ≤ 0.01 for a common tumor. Using these criteria, the increased incidence of tumors in the treated Groups 3, 4 and 5 was not considered significant for all neoplastic lesions, as their p-values were > 0.05 . However, when comparing the tumor incidence in Group 1 to Group 2, the differences were found to be significant ($p = 0.0344$) for combined c-cell adenomas or carcinomas in the thyroid of males if this tumor is classified as a rare tumor. Overall, there was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

Carcinogenicity Summary: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/ kg/ day (feed supplemented with iron). Doses were decreased on Day 29 of the study based upon the recommendations of ExecCAC. Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. No test article-related mortality or moribundity was evident upon histopathological assessment. At

study termination, 72-84% survival was observed in the males and 58-68% survival was observed in the females. There were no statistical differences in survival between the control groups and ICL670-treated dose groups. There were no test article-related effects noted during palpable mass examination. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

Carcinogenicity Conclusions: In conclusion, based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks was not appeared to be carcinogenic at doses up to 60 mg/kg/day in either sex.

Recommendations for further analysis: None.

Labeling Recommendations: None

Addendum/Appendix Listing:

Appendix-1: Exec CAC meeting minutes dated June 5, 2001, page: 65

Appendix-2: List of neoplastic and non-neoplastic lesions, page: 69

Dose-Ranging Study Report: None

CAC Report: None

Alternative Study Protocols and CAC Report: None

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Mouse Carcinogenicity Study Results**

P/T REVIEWER: Tamal K. Chakraborti, Ph.D.

DATE:

IND/NDA: 21-882

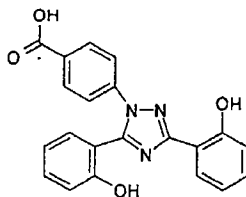
DRUG CODE: ICL670

CAS#: 201530-41-8

DIVISION: HFD-180

DRUG NAME: Exjade[®]/Deferasirox

CHEMICAL STRUCTURE:



SPONSOR: Novartis Pharmaceuticals Corporation, East Hanover, NJ

LABORATORY: Novartis Pharmaceuticals Corp., East Hanover, NJ

CARCINOGENICITY STUDY REPORT DATE: May 24, 2004

THERAPEUTIC CATEGORY: ICL-670 is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis).

PHARMACOLOGICAL CLASSIFICATION: Iron chelator

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): ICL670 was negative in the Ames test and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests.

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MOUSE CARCINOGENICITY STUDY :

MOUSE STUDY DURATION (Weeks): 26 Weeks

STUDY STARTING DATE: October 7, 2002

STUDY ENDING DATE: May 24, 2004

MOUSE STRAIN:

B6.129-Trp53^{tm1} N5 mouse (p53 heterozygous) or C57BL/6/TacfBR-[KO]p53 N5 mouse (p53 heterozygous)

B6.129-Trp53^{tm1} N5 mouse (p53 wild-type) or C57BL/6/TacfBR-[KO]p53 N5 mouse (p53 wild-type)

ROUTE: Oral (gavage)

DOSING COMMENTS: Doses for this study were based on results of a 4-week oral (gavage) range finding study (0170121) in iron supplemented non-iron supplemented (standard diet) C57BL/6 mice at 0, 100, 300, and 600→450 mg/kg/day. In the carcinogenicity study, ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron, Control 1), 0 (standard feed, Control 2), 20 (Group 3), 100 (Group 4) and 200 (Group 5) mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed, Group 6) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron, Group 1), 0 (standard feed, Group 2), 30 (Group 3), 100 (Group 4) and 300 (Group 5) mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, Group 6, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group, Group 8 and 9) were added to the study (Group 8: 0 mg/kg/day and Group 9: 30 mg/kg/day, standard feed). An additional group (Group 7) of p53 heterozygous mice (25/sex) received p-cresidine, and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The dosing volume for all dose groups was 10 ml/kg.

NUMBER OF MICE: 25/sex/group. The study design is shown in the following table (from page 22 of the study report)

Table 3-2 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (heterozygous mice): [] protocol number 0270117					
Group	Number/sex	Animal Numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0 (feed with iron)	0 (feed with iron)
		1026-27	1526-27		
2 Control	25 2 toxicokinetic	2001-25	2501-25	0 (standard feed)	0 (standard feed)
		2026-27	2526-27		
3	25 6 toxicokinetic	3001-25	3501-25	20 (feed with iron)	30 (feed with iron)
		3026-31	3526-31		
4	25 6 toxicokinetic	4001-25	4501-25	100 (feed with iron)	100 (feed with iron)
		4026-31	4526-31		
5	25 6 toxicokinetic	5001-25	5501-25	200 (feed with iron)	300 (feed with iron)
		5026-31	5526-31		
6	25 6 toxicokinetic	6001-25	6501-25	30 (standard feed)	100→60* (standard feed)
		6026-31	6526-31		
7 Positive control	25	7001-25	7501-25	400 p-cresidine (non-supplemented feed)	
8** Control	25 2 toxicokinetic		8501-25	0 (standard feed)	
			8526-27		
9**	25 6 toxicokinetic		9501-25	30 (standard feed)	
			9526-31		

*Dose decrease effective 25-Nov-2002 (day 50) based on the early sacrifice/death of female mice at 100 mg/kg/day.

**Additional female dose groups were added because of early sacrifice/death in the group 6 female mice.

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. Animals in groups 1-7 were acclimated to fasting procedures for 2 weeks prior to the start of dosing. Animals in groups 8-9 were acclimated to fasting procedures for approximately 1 week prior to dosing.

In order to facilitate on-line data collection, p53 heterozygous mice were assigned to — protocol number 0270117 (groups 1-7) or — protocol number 0270117f (groups 8 and 9). The wild type mice were assigned to — protocol number 0270117w.

Table 3-3 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (wild type mice): L J ₁ protocol number 0270117w					
Group	Number/sex	Animal numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0	0
		1026-27	1526-27	(feed with iron)	(feed with iron)
2 Control	25 2 toxicokinetic	2001-25	2501-25	0	0
		2026-27	2526-27	(standard feed)	(standard feed)
3	25 6 toxicokinetic	3001-25	3501-25	200	300
		3026-31	3526-31	(feed with iron)	(feed with iron)

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. All animals were acclimated to fasting procedures for 2 weeks prior to the start of dosing.

MOUSE DOSE LEVELS (mg/kg/day):

p53 Heterozygous Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)
- Group 4: 100 mg/kg/day (feed + iron)
- Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)
- Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)
- Group 7: p-Cresidine (400 mg/kg/day, oral)
- Group 8: 0 mg/kg/day (female, standard feed)
- Group 9: 30 mg/kg/day (female, standard feed)

Wild Type Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible):

MTD. Doses for this study (0170121) were based on results of a 4-week oral (gavage) range finding toxicity study in C57BL/6 mice. In this dose ranging study, ICL670 was administered in aqueous 0.5% Klucel to 4 groups of mice fed an iron supplemented diet at doses of 0, 100, 300, and 600→450 mg/kg/day. ICL670 was also administered to 3 groups of mice fed a standard diet at doses of 0, 100 and 600 mg/kg/day. The doses for the current study were selected based on

the CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002, and November 14, 2002, Appendix-3, 4, and 5, respectively).

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Yes. The doses were selected as per ExecCAC recommendations (ExecCAC meeting minutes dated November 14, 2002, Appendix-5).

MOUSE CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Negative.

MOUSE TUMOR FINDINGS: There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation. As expected, the administration of p-cresidine (positive control) caused proliferative changes notably in the urinary bladder of p53 heterozygous mice.

MOUSE STUDY COMMENTS: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine, and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The dosing volume for all dose groups was 10 ml/kg. The doses for the current study were selected in accordance with CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002, and November 14, 2002, Appendix-3, 4, and 5, respectively). Overall, the design of the study appears to be appropriate and was developed as per PTCC and ExecCAC recommendations. It is to be mentioned here that the sponsor did not conduct statistical analysis of tumor incidences. The sponsor provided limited historical control data for p53 heterozygous and wild type mice for this testing facility from three different studies conducted during the period of 1999-2003.

The statistician has stated in his review (dated August 11, 2005) that some organs and tissues were not examined. This is to be noted here that the sponsor has conducted histopathologic examinations of most of the protocol specified tissues. However, in some cases, some protocol specified tissues were not examined. This does not appear to have any significant impact on the interpretation of the study results for the following reasons: a) small numbers of protocol specified tissues were not examined and b) in most cases, the number of tissues “not examined” were distributed across all dose groups.

COVER SHEET FOR CARCINOGENICITY STUDY IN THE MOUSE

1. **Study Number:** 0270117
2. **Name of Laboratory:** Novartis Pharmaceuticals, East Hanover, NJ
3. **Strain:**

B6.129-Trp53^{tm1} N5 mouse (p53 heterozygous) or C57BL/6/Tac^fBR-[KO]p53 N5 mouse (p53 heterozygous)

B6.129-Trp53^{tm1} N5 mouse (p53 wild-type) or C57BL/6/Tac^fBR-[KO]p53 N5 mouse (p53 wild-type)

4. **No./Sex/Group:** 25
5. **Doses (0, L, M, and H):**

p53 Heterozygous Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)
- Group 4: 100 mg/kg/day (feed + iron)
- Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)
- Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)
- Group 7: p-Cresidine (400 mg/kg/day, oral)
- Group 8: 0 mg/kg/day (female, standard feed)
- Group 9: 30 mg/kg/day (female, standard feed)

Wild Type Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). The doses are shown in the following table (from page 22 of the study report)

Table 3-2 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (heterozygous mice): C I protocol number 0270117					
Group	Number/sex	Animal Numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25 1026-27	1501-25 1526-27	0 (feed with iron)	0 (feed with iron)
2 Control	25 2 toxicokinetic	2001-25 2026-27	2501-25 2526-27	0 (standard feed)	0 (standard feed)
3	25 6 toxicokinetic	3001-25 3026-31	3501-25 3526-31	20 (feed with iron)	30 (feed with iron)
4	25 6 toxicokinetic	4001-25 4026-31	4501-25 4526-31	100 (feed with iron)	100 (feed with iron)
5	25 6 toxicokinetic	5001-25 5026-31	5501-25 5526-31	200 (feed with iron)	300 (feed with iron)
6	25 6 toxicokinetic	6001-25 6026-31	6501-25 6526-31	30 (standard feed)	100→60* (standard feed)
7 Positive control	25	7001-25	7501-25	400 p-cresidine (non-supplemented feed)	
8** Control	25 2 toxicokinetic		8501-25 8526-27	0 (standard feed)	
9**	25 6 toxicokinetic		9501-25 9526-31	30 (standard feed)	

*Dose decrease effective 25-Nov-2002 (day 50) based on the early sacrifice/death of female mice at 100 mg/kg/day.

**Additional female dose groups were added because of early sacrifice/death in the group 6 female mice.

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. Animals in groups 1-7 were acclimated to fasting procedures for 2 weeks prior to the start of dosing. Animals in groups 8-9 were acclimated to fasting procedures for approximately 1 week prior to dosing.

In order to facilitate on-line data collection, p53 heterozygous mice were assigned to - protocol number 0270117 (groups 1-7) or - protocol number 0270117f (groups 8 and 9). The wild type mice were assigned to - protocol number 0270117w.

Table 3-3 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (wild type mice): [protocol number 0270117w					
Group	Number/sex	Animal numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0 (feed with iron)	0 (feed with iron)
		1026-27	1526-27		
2 Control	25 2 toxicokinetic	2001-25	2501-25	0 (standard feed)	0 (standard feed)
		2026-27	2526-27		
3	25 6 toxicokinetic	3001-25	3501-25	200 (feed with iron)	300 (feed with iron)
		3026-31	3526-31		

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. All animals were acclimated to fasting procedures for 2 weeks prior to the start of dosing.

6. **Basis for Dose Selection Stated:** Yes. Maximum tolerated dose (MTD). Doses were based on the results of a 4-week oral (gavage) range finding toxicity study in C57BL/6 mice (Study 0170121) and as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002, and November 14, 2002, Appendix-3, 4, and 5, respectively).
7. **Interim Sacrifice:** None
8. **Total Duration (Weeks):** 26 Weeks
9. **Week/Site for First Tumor:** The sponsor did not provide chronological tumor data.
10. **Number Alive at Termination:** The following table shows the summary of mortality in Groups 1-9 in males and females.

MALE								
GROUPS*								
1	2	3	4	5	6	7		
0	0	2	2	0	1	2		
FEMALES								
GROUPS								
1	2	3	4	5	6	7	8	9
2	1	1	3	8**	6**	0	0	1

**: Deaths considered treatment-related

*:

p53 Heterozygous Mice

Group 1: Control 1 (feed + iron)

Group 2: Control 2 (standard feed)

Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)

Group 4: 100 mg/kg/day (feed + iron)

Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)

Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)

Group 7: p-Cresidine (400 mg/kg/day, oral)

Group 8: 0 mg/kg/day (female, standard feed)

Group 9: 30 mg/kg/day (female, standard feed)

Wild Type Mice

Group 1: Control 1 (feed + iron)

Group 2: Control 2 (standard feed)

Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

The following tables (from page 32 and 33 of the study report) show the mortality data in different dose groups.

For heterozygous mice supplemented with iron:

Table 4-1 Summary of early death/sacrifice (heterozygous mice with iron)

Group:	Males Dose (mg/kg/day)				Females Dose (mg/kg/day)			
	1 (0)	3 (20)	4 (100)	5 (200)	1 (0)	3 (30)	4 (100)	5 (300)
Week								
26	0	2	2	0	2	1	3	8*

*Five of the eight early deaths/sacrifices were considered test article-related.

For heterozygous mice fed a standard diet:

Table 4-2 Summary of early death/sacrifice (heterozygous mice, no iron)

Group:	Males Dose (mg/kg/day)			Females Dose (mg/kg/day)		
	2 (0)	6 (30)	7 (400 p-cresidine)	2 (0)	6 (100→60)	7 (400 p-cresidine)
Week 26	0	1	2	1	6*	0

*All of the six early deaths/sacrifices were considered test article-related.

Table 4-3 Summary of early sacrifice/death (heterozygous mice, no iron)

Females	
Group:	8
Dose:	0 mg/kg/day
Week 26	0
	9
	30 mg/kg/day
	1

Animal no. 9503 was sacrificed on day 19. Based on the macroscopic and microscopic findings the cause of moribundity was misgavage.

For the wild type mouse supplemented with iron:

Table 4-4 Summary of early deaths/sacrifices (wild type with iron)

Group:	Males Dose (mg/kg/day)			Females Dose (mg/kg/day)		
	1 (0)	2 (0)	3 (200)	1 (0)	2 (0)	3 (300)
Week 26	0	0	1*	0	0	1

*Moribund sacrifice considered test article-related.

11. **Statistical Methods Used:** In-life data (body weight, food consumption, clinical pathology and organ weights): Means and standard deviations for body weight and food consumption determinations were calculated and an Analysis of Variance (ANOVA) was performed. The ANOVA was followed by a Bartlett's test for homogeneity of variances. When the variances were homogeneous, Dunnett's t-test was employed to determine the statistical significance between control and treated groups. When the variances were not homogeneous, a modified t-test was used to determine which groups were statistically different from the controls. Means and standard deviations were calculated for the clinical pathology and organ weight data. The sponsor did not perform statistical analysis of tumor incidences.

12. **Attach Tumor and Non-Tumor Data for Each Tissue:** List of neoplastic and non-neoplastic lesions is attached in Appendix-6.

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CARCINOGENICITY:

Study Title: 26-Week Oral (Gavage) Carcinogenicity Study in p53 Heterozygous Mice

Key Study Findings:

Treatment-related moribundity/mortality occurred in one p53 heterozygous female at 300 mg/kg/day receiving the iron supplemented diet and one p53 heterozygous female at 100→60 mg/kg/day receiving the standard rodent diet. Nine other p53 mice (4 females, supplemented with dietary iron at 300 mg/kg/day and 5 females, without dietary iron supplementation at 100 mg/kg/day) were also considered test article-related. One test article-related moribundity was also observed in a high-dose wild type male at 200 mg/kg/day with dietary iron.

Test article-related decreases in mean body weight were observed in p53 heterozygous mice supplemented with iron at 200 mg/kg/day (males) and 300 mg/kg/day (females), in p53 heterozygous mice receiving standard feed in the males at 30 mg/kg/day and in the females at 100→60 mg/kg/day and in wild type mice supplemented with iron in males at 200 mg/kg/day and females at 300 mg/kg/day.

Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female mouse at 300 mg/kg/day.

There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation.

The major effects observed with *p*-cresidine, the positive control, included proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%).

Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were predominantly observed in non-supplemented mice.

Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Study Number: 0270117

Volume #, and Page #: Page 1 of Report 0270117

Conducting Laboratory and Location: Novartis Pharmaceuticals Inc., East Hanover, NJ

Date of Study Initiation: October 7, 2002

Date of Study Completion: May 24, 2004

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, τ $\uparrow$

CAC Concurrence: Yes. Exec CAC meeting minutes dated August 21, 2001 (Appendix-3), September 19, 2001 (Appendix-4) and November 14, 2002 (Appendix-5) are attached.

Study Type: Transgenic assay

Species/Strain:

B6.129-Trp53^{tm1} N5 mouse (p53 heterozygous) or C57BL/6/TacfbR-[KO]p53 N5 mouse (p53 heterozygous)

B6.129-Trp53^{tm1} N5 mouse (p53 wild-type) or C57BL/6/TacfbR-[KO]p53 N5 mouse (p53 wild-type)

Number/Sex/Group; Age at Start of Study: 25/sex/group; Age: 11 weeks old

Body Weight: Males: 22 to 32 g; Females: 17 to 25 g

Animal Housing: Animals were housed singly in solid bottom cage.

Formulation/Vehicle: Suspension in 0.5% (w/v) Klucel (type HF), NF

Drug Stability/Homogeneity: Based on stability data, ICL670 in 0.5% Klucel, 0.75 mg/mL to 100 mg/mL, was found to be stable for use for at least τ $\uparrow$ at 6°C and at least τ $\uparrow$ stirring at room temperature. Aqueous 0.5% Klucel was found to be suitable for use for at least τ $\uparrow$ stirring at room temperature and at least τ $\uparrow$ stored at 6°C. The uniformity of dosing formulations was also confirmed. Samples of the formulations for Weeks 1, 8, 13, and 26 were tested, and the concentrations were found to be τ $\uparrow$ of target concentrations. Six control samples were also tested, and no ICL670 was detected.

Methods:

Doses: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a

suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The dosing volume for all dose groups was 10 ml/kg. The doses for the current study were selected as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002, and November 14, 2002, Appendix-3, 4, and 5, respectively). Doses are shown below.

p53 Heterozygous Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)
- Group 4: 100 mg/kg/day (feed + iron)
- Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)
- Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)
- Group 7: p-Cresidine (400 mg/kg/day, oral)
- Group 8: 0 mg/kg/day (female, standard feed)
- Group 9: 30 mg/kg/day (female, standard feed)

Wild Type Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

The following table presents the study design (from page 19 of sponsor's report).

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Table 3-2 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (heterozygous mice): [] protocol number 0270117					
Group	Number/sex	Animal Numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0 (feed with iron)	0 (feed with iron)
		1026-27	1526-27		
2 Control	25 2 toxicokinetic	2001-25	2501-25	0 (standard feed)	0 (standard feed)
		2026-27	2526-27		
3	25 6 toxicokinetic	3001-25	3501-25	20 (feed with iron)	30 (feed with iron)
		3026-31	3526-31		
4	25 6 toxicokinetic	4001-25	4501-25	100 (feed with iron)	100 (feed with iron)
		4026-31	4526-31		
5	25 6 toxicokinetic	5001-25	5501-25	200 (feed with iron)	300 (feed with iron)
		5026-31	5526-31		
6	25 6 toxicokinetic	6001-25	6501-25	30 (standard feed)	100→60* (standard feed)
		6026-31	6526-31		
7 Positive control	25	7001-25	7501-25	400 p-cresidine (non-supplemented feed)	
8** Control	25 2 toxicokinetic		8501-25	0 (standard feed)	
			8526-27		
9**	25 6 toxicokinetic		9501-25	30 (standard feed)	
			9526-31		

*Dose decrease effective 25-Nov-2002 (day 50) based on the early sacrifice/death of female mice at 100 mg/kg/day.

**Additional female dose groups were added because of early sacrifice/death in the group 6 female mice.

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. Animals in groups 1-7 were acclimated to fasting procedures for 2 weeks prior to the start of dosing. Animals in groups 8-9 were acclimated to fasting procedures for approximately 1 week prior to dosing.

In order to facilitate on-line data collection, p53 heterozygous mice were assigned to [] protocol number 0270117 (groups 1-7) or [] protocol number 0270117f (groups 8 and 9). The wild type mice were assigned to [] protocol number 0270117w.

Table 3-3 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (wild type mice): [] protocol number 0270117w					
Group	Number/sex	Animal numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0	0
		1026-27	1526-27	(feed with iron)	(feed with iron)
2 Control	25 2 toxicokinetic	2001-25	2501-25	0	0
		2026-27	2526-27	(standard feed)	(standard feed)
3	25 6 toxicokinetic	3001-25	3501-25	200	300
		3026-31	3526-31	(feed with iron)	(feed with iron)

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. All animals were acclimated to fasting procedures for 2 weeks prior to the start of dosing.

Basis of dose selection: Doses for this study (0170121) were based on results of a 4-week oral (gavage) range finding toxicity study in C57BL/6 mice. In this dose ranging study, ICL670 was administered in aqueous 0.5% Klucel to 4 groups of mice fed an iron supplemented diet at doses of 0, 100, 300, and 600→450 mg/kg/day. ICL670 was also administered to 3 groups of mice fed a standard diet at doses of 0, 100 and 600 mg/kg/day. The doses for the current study were selected based on the CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002, and November 14, 2002, Appendix-3, 4, and 5, respectively).

Route of administration: Oral (gavage)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Satellite PK: Yes. Blood samples were collected from satellite animals at approximately 0.5, 2 and 6 hours postdose during Week 23 to determine the toxicokinetic (TK) profile of ICL670.

Deviations from original study protocol: On January 18, 2003 (Day 31), the Group 8 female toxicokinetic control dose group (animal nos. 8526 and 8527) were inadvertently dosed with the 3 mg/ml formulation intended for the Group 9 study animals (30

mg/kg/day). This dosing error did not appear to have any significant impact on the study or the TK results, since no ICL670 was detected during the Week 23 TK sampling.

Statistical methods: In-life data (body weight, food consumption, clinical pathology and organ weights): Means and standard deviations for body weight and food consumption determinations were calculated and an Analysis of Variance (ANOVA) was performed. The ANOVA was followed by a Bartlett's test for homogeneity of variances. When the variances were homogeneous, Dunnett's t-test was employed to determine the statistical significance between control and treated groups. When the variances were not homogeneous, a modified t-test was used to determine which groups were statistically different from the controls. Means and standard deviations were calculated for the clinical pathology and organ weight data. The sponsor did not perform statistical analysis of tumor incidences.

Observations and Times:

Clinical Signs: Clinical signs were observed once daily.

Body Weights: Body weights were recorded once during pretest, and once weekly during Weeks 1 to 14; every two weeks from Weeks 16 to 26.

Food Consumption: Food consumption was recorded once weekly during Weeks 1 to 14, every two weeks from Weeks 16 to 26.

Ophthalmoscopy: Ophthalmoscopic examinations (using a Fison Indirect Ophthalmoscope) were conducted on all animals during pretest and on all surviving animals during Weeks 4, 15, and 26.

Palpable Mass Examination: Palpable mass examinations were conducted on all main study animals beginning Week 2 and every two weeks thereafter.

Clinical Pathology: Clinical pathology assessments (hematology and serum chemistry) were conducted on the last 10 surviving animals/sex/group at scheduled necropsy.

Plasma and Tissue Iron Analysis: Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) were determined in the serum. In addition, liver (section of right lobe) and kidney (right pole) were collected after exsanguination from the last 10 surviving animals/sex/group at the terminal sacrifice for the determination of iron content by means of γ following mineralization of the tissue samples.

Organ Weights: The following organs were weighed (marked with "W" in the table below) from all animals: brain, heart, kidney, liver, spleen, testis and thymus.

Histopathology: The tissues specified in the following list (from page 28 of the study report) were examined for histopathology:

Tissue list: for Collection and/or Processing (P)

	P	adrenal			nasal passage
	P	aorta		P	ovary
	P	blood and bone marrow smears		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
W	P	brain		P	pituitary
	P	cecum		P	preputial gland
	P	cervix		P	prostate
	P	ditoral gland		P	rectum
	P	colon		P	salivary gland
	P	duodenum		P	sciatic nerve
	P	epididymis		P	seminal vesicle
	P	esophagus		P	skeletal muscle
	P	eye		P	skin
	P	femur/tibia		P	spinal cord
	P	gallbladder	W	P	spleen
	P	harderian gland		P	sternum
W	P	heart		P	stomach
	P	ileum	W	P	testis
	P	jejunum	W	P	thymus
W	P	kidney		P	thyroid
	P	lacrimal gland		P	tongue
W	P	liver		P	trachea
	P	lung		P	urinary bladder
	P	lymph node – bronchial		P	uterus
	P	lymph node – mandibular		P	vagina
	P	lymph node – mesenteric		P	macroscopic lesions
	P	mammary gland			animal identification (right ear, left ear or tail)

Toxicokinetics: Blood was collected from all TK animals during Week 23. For the control groups, surviving TK animals were bled at approximately 2 hours post dose. For ICL670-treated animals, surviving animals were bled at approximately 0.5, 2 and 6 hours postdose.

Results:

Mortality:

Heterozygous mice fed an iron-supplemented diet: Test article-related mortality was observed in one high dose female at 300 mg/kg/day supplemented with iron (animal no. 5518 on Day 124). The cause of death was marked erosion of the glandular stomach. In addition, four additional p53 heterozygous mice that died or were sacrificed early (animal nos. 5501, 5504, 5505 and 5509 treated at 300 mg/kg/day, all supplemented with iron) between Days 56 and 170 were also considered test article-related. The cause of death could not be confirmed upon histopathological assessment; however a relationship to the treatment was not ruled out. In animal nos. 5501, 5504 and 5509 relevant clinical signs which were observed before sacrifice included severe decreased locomotor activity, cold to touch, hunched posture, ptosis, reduced feces and labored respiration. The remaining heterozygous mice supplemented with iron that died or were sacrificed early were considered to be due to gavage accidents (animal nos. 1505, 4007, 4016, 5519 and 5524), lymphoma (animal nos. 3021, 3023 and 3505), osteosarcoma (animal nos. 1506, 5516), mammary adenocarcinoma (animal no. 4512) or arteritis (animal no. 4522). One female (animal no. 4502 treated at 100 mg/kg/day) was sacrificed on Day 183 due to an apparent injury which caused hindlimb paralysis. A gross lesion on the leg was collected at necropsy, however there were no microscopic findings. Although the cause of death of this animal could not be determined; however, it considered to be due to injury and therefore was considered treatment-related. The following table (from page 32 of the study report) shows the summary of early death/sacrifice in heterozygous mice fed on iron-supplemented diet.

For heterozygous mice supplemented with iron:

Table 4-1 Summary of early death/sacrifice (heterozygous mice with iron)

Group:	Males Dose (mg/kg/day)				Females Dose (mg/kg/day)			
	1 (0)	3 (20)	4 (100)	5 (200)	1 (0)	3 (30)	4 (100)	5 (300)
Week								
26	0	2	2	0	2	1	3	8*

*Five of the eight early deaths/sacrifices were considered test article-related.

For heterozygous mice fed a standard diet: Treatment-related mortality was observed in one female at 100 mg/kg/day (animal no. 6525 on Day 22). Relevant clinical signs preceding sacrifice included cold to touch, thin appearance, moderate decreases in locomotor activity and moderate ataxia. The cause of death was considered to be stomach ulceration. In addition, five other females that died or were sacrificed early (Animal nos. 6504, 6505, 6514, 6520 and 6523) between Days 36 and 42 of the study were also considered treatment-related. However, the cause of death was not evident upon histopathological examinations. Clinical signs for these five animals included decreased locomotor activity, dehydration, thin appearance, hunched posture, muscle tremors and/ or labored respiration prior to death or sacrifice. In addition, one male (animal no. 6002) treated at 30 mg/kg/day was sacrificed moribund on Day 157. The

cause of death was considered to be due to inflammation of the secondary sex organs surrounding the lower urinary tract and was not considered treatment-related. One control female (animal no. 2518) was sacrificed on Day 183. The cause of death was arteritis. The following table (from page 32 of the study report) shows the mortality in heterozygous mice fed with standard diet.

For heterozygous mice fed a standard diet:

Table 4-2 Summary of early death/sacrifice (heterozygous mice, no iron)

	Males			Females		
	Dose (mg/kg/day)			Dose (mg/kg/day)		
Group:	2 (0)	6 (30)	7 (400 p-cresidine)	2 (0)	6 (100→60)	7 (400 p-cresidine)
Week						
26	0	1	2	1	6*	0

*All of the six early deaths/sacrifices were considered test article-related.

p-Cresidine mice: Two males died during the treatment phase. Animal no. 7009 was found dead on Day 105 and animal no. 7015 was found dead on Day 175. These animals exhibited slightly decreased locomotor activity (7015) and animal 7009 had a visible swelling/mass. The cause of death in these animals was considered to be due to skin carcinoma (animal no. 7009) or urinary bladder carcinoma (animal no. 7015). The above table (from page 33 of the study report) shows summary of mortality in p-cresidine animals.

Heterozygous mice fed on a standard diet (Additional Groups 8 and 9): Animal no. 9503 was sacrificed on Day 19. Based on the macroscopic and microscopic findings, the cause of death was considered to be due to gavage accident. The following table (from page 33 of the study report) shows the mortality in Groups 8 and 9.

Table 4-3 Summary of early sacrifice/death (heterozygous mice, no iron)

	Females	
	Group:	8
Dose:	0 mg/kg/day	30 mg/kg/day
Week		
26	0	1

Wild type mouse fed on an iron-supplemented diet: Animal no. 3021 treated at 200 mg/kg/day was sacrificed moribund on Day 114. The cause of death was considered to be due to stomach and kidney lesions which were attributable to treatment. This animal also had lesions in the lung which suggested that misgavage may also have contributed to

the moribundity of this animal. One female (animal no. 3514 treated at 300 mg/kg/day) was sacrificed for humane reasons on Day 85. The cause of death was considered as arteritis. This animal exhibited decreased locomotor activity, hunched posture, ptosis, reduced feces and piloerection of the fur and the female exhibited head tilt, circular movements, impaired righting reflex, ataxia and a thin appearance. The following table (from page 34 of the study report) shows the mortality in wild type mice fed on iron-supplemented diet.

For the wild type mouse supplemented with iron:

Table 4-4 Summary of early deaths/sacrifices (wild type with iron)

	Males			Females		
	Dose (mg/kg/day)			Dose (mg/kg/day)		
Group:	1	2	3	1	2	3
	(0)	(0)	(200)	(0)	(0)	(300)
Week						
26	0	0	1*	0	0	1

*Moribund sacrifice considered test article-related.

The following table shows the summary of mortality in Groups 1-9 in males and females.

MALE								
GROUPS*								
1	2	3	4	5	6	7		
0	0	2	2	0	1	2		
FEMALES								
GROUPS								
1	2	3	4	5	6	7	8	9
2	1	1	3	8**	6**	0	0	1

** : Deaths considered treatment-related

* :

p53 Heterozygous Mice

Group 1: Control 1 (feed + iron)

Group 2: Control 2 (standard feed)

Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)

Group 4: 100 mg/kg/day (feed + iron)

Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)

Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)

Group 7: p-Cresidine (400 mg/kg/day, oral)

Group 8: 0 mg/kg/day (female, standard feed)
 Group 9: 30 mg/kg/day (female, standard feed)
Wild Type Mice

Group 1: Control 1 (feed + iron)
 Group 2: Control 2 (standard feed)
 Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

Clinical Signs: Treatment-related clinical signs observed in animals that were died or sacrificed due to moribund conditions generally included severely decreased locomotor activity, cold to touch, hunched posture, ptosis, reduced feces, labored respiration, dehydration, thin appearance and/or muscle tremors. For the *p*-cresidine mice, all animals exhibited slightly decreased locomotor activity. Additional signs included ptosis, wet stains on fur and a thin appearance.

Body Weights:

For heterozygous mice supplemented with iron (Groups 1, 3, 4 and 5):

The mean initial and final body weights of control (Group 1) males were 24.2 and 37.8 g, respectively. The mean initial and final body weights of control (Group 1) females were 19.1 and 30.9 g, respectively. There were no test article-related effects on mean body weight in either sex at doses up to 100 mg/kg/day. Test article-related decreases in mean body weight were observed at 200 mg/kg/day in males (6-7%) and 300 mg/kg/day in females (6-8%). Body weights in Groups 1, 3, 4 and 5 are shown in the following table.

Day	Males (Iron supplemented)				Females (Iron supplemented)			
	Group 1	Group 3	Group 4	Group 5	Group 1	Group 3	Group 4	Group 5
-3	24.2	24.3	24.3	24.5	19.1	18.9	19.1	18.7
15	28.2	28.4	28.2	28.2	23.2	22.5	22.4	22.8
29	29.3	29.3	29.6	28.9	23.7	23.2	23.2	23.0
92	35.0	34.5	34.9	32.8	28.3	26.8	26.8	26.6
176	37.8	38.4	39.6	37.3	30.9	31.4	30.9	28.3
Body Weight (% of Control)								
176	100	101.6%	104.8%	98.7%	100%	101.6%	100%	91.6%

For heterozygous mice fed a standard diet (Groups 2, 6 and 7): The mean initial and final body weights of control (Group 2) males were 24.3 and 36.9 g, respectively. The mean initial and final body weights of control (Group 2) females were 19.0 and 28.7 g, respectively. There were no test article-related effects on mean body weight in females at 30 mg/kg/day. Treatment-related decreases in mean body weight were observed in

males (5-8%) at 30 mg/kg/day and in females (5-8%) at 100→60 mg/kg/day. The following table shows the body weight data for Groups 2, 6 and 7.

Day	Males (Iron supplemented)			Females (Iron supplemented)			
	Group 2	Group 6	Group 7	Group 2	Group 6	Group 7	
-3	24.3	24.5	19.0	19.0	18.7	19.0	
15	27.7	27.7	22.3	22.9	22.7	22.3	
29	29.1	28.6	22.4	23.9	23.2	22.4	
92	33.2	30.8	26.0	26.4	24.3	23.2	
176	36.9	34.5	26.4	28.7	27.6	23.4	

For the wild type mouse supplemented with iron (Groups 1, and 3): The mean initial and final body weights of control (Group 1) males were 24.5 and 41.5 g, respectively. The mean initial and final body weights of control (Group 1) females were 20.0 and 36.5 g, respectively. There were no test article-related decreases in mean body weight in males at 200 mg/kg/day. In the females at 300 mg/kg/day, slight, statistically significant test article-related decreases (up to 12%) in mean body weight were observed starting on Day 29 and persisted through Day 176.

Day	Males (Iron supplemented)			Females (Iron supplemented)			
	Group 1	Group 3		Group 1	Group 3		
-3	24.5	24.6		20.0	19.9		
15	29.0	29.6		24.8	24.3		
29	30.3	30.9		26.3	29.2		
92	36.8	36.2		29.3	29.3		
176	41.5	40.2		36.5	32.7		

For the *p*-cresidine mice: The *p*-cresidine mice exhibited decreases in mean body weight in both sexes at 400 mg/kg/day throughout the treatment period (decreases of 4- 28% in the males and 4-18% in the females).

Food Consumption:

For heterozygous mice supplemented with iron (Groups 1, 3, 4 and 5): The mean initial and final food consumption in control (Group 1) males were 6.9 and 4.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 8.5 and 5.4 g/animal/day, respectively. There were no significant treatment-related effects.

For heterozygous mice fed a standard diet: The mean initial and final food consumption in control (Group 2) males were 5.6 and 3.6 g/animal/day, respectively. The mean initial

and final food consumption in control females were 6.2 and 4.0 g/animal/day, respectively. There were no significant treatment-related effects.

For the wild type mouse mice supplemented with iron: The mean initial and final food consumption in control (Group 1) males were 7.8 and 5.7 g/animal/day, respectively. The mean initial and final food consumption in control females were 8.2 and 6.7 g/animal/day, respectively. There were no test article-related effects.

For the *p*-cresidine mice: Mice treated with *p*-cresidine displayed decreases in food consumption throughout the treatment period.

Ophthalmology: Ophthalmoscopic examinations performed during Week 4 of the study revealed no treatment-related changes. However, ophthalmoscopic examinations performed during Week 15 of the study revealed treatment-related bilateral cataracts in Group 5 (feed supplemented with iron, 200 mg/kg/day for males and 300 mg for females) mice [two of 25 males at 200 mg/kg/day (animal nos. 5020 and 5021 and 2 of 22 females at 300 mg/kg/day (animal nos. 5501 and 5518)]. Ophthalmoscopically, these cataracts were characterized as total opacification of the lens fibers (mature cataracts).

At Week 26, several other mice from Group 5 were similarly affected (4 of 25 males, animal nos. 5001, 5007, 5020 and 5021 and 4 of 17 females, animal nos. 5508, 5511, 5512 and 5514). The lenticular change was identical to those seen earlier (bilateral mature cataracts). At this time, one female (1 of 17, animal no. 5513) and two males (2 of 25, animal nos. 5014 and 5022) of the heterozygous Group 5 mice had incipient/early cataracts. All affected mice (with the exception of the mice affected with incipient/early cataracts) had a visual deficit (blindness). In addition, at Week 26, one wild type female mouse (animal no. 3507 at 300 mg/kg/day) was also observed with bilateral mature cataracts.

Palpable Mass: There was no apparent increase in the incidence of palpable masses observed during the course of the study or prior to scheduled necropsy. A summary of the palpable mass incidence at the last scheduled necropsy is presented in the following table (page 37 and 38 of the study report).

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Table 4-5 Palpable mass incidence in heterozygous mice

Group Dose (mg/kg/day)	Males	Females
1 (0) with iron	0/25	1/24
2 0 (without iron)	0/25	0/25
3 20 / 30 (with iron)	0/23	0/24
4 100 (with iron)	0/23	0/23
5 200 / 300 (with iron)	0/25	0/17
6 30 / 100→60 (without iron)	1/24	0/19
7 400 p-cresidine (without iron)	0/23	0/25
8 0 (without iron)	NA	0/25
9 30 (without iron)	NA	0/24

Table 4-6 Palpable mass incidence in wild type mice

Group Dose (mg/kg/day)	Males	Females
1 0 (with iron)	0/25	0/25
2 0 (without iron)	0/25	0/25
3 200 / 300 (with iron)	0/24	0/24

Hematology: No significant treatment-related changes were observed.

Serum Iron Analysis: Test article-related serum chemistry alterations in the iron-supplemented p53 heterozygous mice included increased iron in both sexes (118-176% of control in males, 127-189% of control, control male = 128 µg/dl; control female = 129 µg/dl) at doses ≥100 mg/kg/day, increased total iron binding capacity (TIBC) in males (104-118% of control, control 313 µg/dl) at doses 200 mg/kg/day and in females (103-141% of control, control = 343 µg/dl) at 300 mg/kg/day, and decreased unsaturated iron binding capacity (UIBC) in males (73-95% of control, control = 187 µg/dl) at 200 mg/kg/day. In the heterozygous mice fed a standard diet treatment-related effects were limited to increased iron (iron: 182-187% of control, control =

104-105 µg/dl) and decreased UIBC concentrations (63-84% of control, control = 231-242 µg/dl) in both sexes at 30 mg/kg/day (iron: 187% of control, control = 104 µg/dl) in males and in females at 100→60 mg/kg/day. In the iron supplemented wild type mice, effects were also limited to increased iron (148-159% of control, control = 137 µg/dl for males and 154 µg/dl for females) and increased TIBC (116-119% of control, control = 342 µg/dl) concentrations in males at 200 mg/kg/day and females at 300 mg/kg/day.

Tissue Iron Analysis: Iron supplementation of the p53 heterozygous control mice produced increases (approximately 30-50%) in liver and kidney iron content when compared to the control mice fed the standard rodent diet. Iron supplementation of the wild type control mice also produced increases in mean liver iron content (approximately 40- 50%) and mean kidney iron content (approximately 30%) in males.

In the iron supplemented p53 heterozygous mice, test article-related decreases in mean liver iron content (approximately 74-81%) and kidney iron content (approximately 40-55%) were noted in both sexes at doses ≥100 mg/kg/day when compared to iron supplemented controls as well as unsupplemented controls (decreases of 50-70% in the liver and 20-30% in the kidney). At doses of 20 mg/kg/day (males) and 30 mg/kg/day (females), decreases in mean liver and kidney iron content were only observed when compared to the iron supplemented controls (liver content was decreased by approximately 20- 30% and mean kidney iron content was decreased by approximately 30% for males and 10% for females).

In the p53 heterozygous mice receiving standard/unsupplemented diet, treatment-related decreases (approximately 51-64%) in mean liver iron content were observed in males at 30 mg/kg/day and in females at 100→60 and 30 mg/kg/day. Mean kidney iron content was also decreased by about 32% in the males at 30 mg/kg/day and by about 24-33% in females at doses of 100→60 and 30 mg/kg/day.

In the wild type mice, receiving iron-supplemented diet, treatment-related decreases in mean liver iron content were observed in both sexes (by approximately 71-77% when compared to the iron supplemented controls and by approximately 50% when compared to the unsupplemented controls). Mean kidney iron content also appeared to be decreased by about 60% in both sexes when compared to iron-supplemented controls and by about 40-57% when compared to the unsupplemented controls.

Organ Weights: Treatment-related organ weight changes were observed in the kidney and thymus. Kidney weights were increased in iron-supplemented p53 heterozygous and wild type males and females treated at 200 (107% of control, control = 0.430 g) and 300 mg/kg/day (108% of control, control = 108 g), respectively, and in non-supplemented p53 heterozygous females treated at 100→60 mg/kg/day (109% of control) when compared to control (0.308 g). Thymus weights were decreased in iron-supplemented, p53 heterozygous males and females treated at 200 (86% of control, control = 0.029 g) and 300 mg/kg/day (72% of control, control = 0.043 g), respectively, when compared to control.

Macroscopic Examinations: Overall treatment-related macroscopic changes included eye opacity, pale discoloration of the kidneys, and dark foci in the stomach. In addition, *p*-cresidine-treated p53 heterozygous male mice had an increased incidence of thickening of the bladder and both sexes had a variety of macroscopic renal abnormalities, including discoloration, cystic change and pitted surface.

Histopathology:

Non-Neoplastic: Treatment-related non-neoplastic findings were observed in the eye (cataract at 200 and 300 mg/kg/day in iron-supplemented p53 heterozygous males and females, respectively; at 100→60 mg/kg/day in unsupplemented heterozygous female mice, and at 300 mg/kg/day in wild type females), kidney (diffuse tubular vacuolation, loss of normal focal tubular vacuolation, increased basophilic tubules), liver (biliary hyperplasia/periportal inflammation), stomach (increased severity of erosion in the glandular stomach and hyperkeratosis and ulcer in the non-glandular stomach), and skeletal muscle (degeneration/regeneration). The following table (from page 40 of the study report) shows the selected treatment-related histopathology findings in different groups.

Table 4-7 Selected test article-related findings

	p53 heterozygous + iron		p53 heterozygous no iron		Wild type + iron	
	Dose*		Dose*		Dose*	
	M	F	M	F	M	F
Eye: Cataract	200	300	-	100→60	-	300
Kidney:						
Diffuse tubular vacuolation	-	300	30	100	200	-
Loss of normal focal tubular vacuolation	≥100	-	30	-	200	-
Increased basophilic tubules	-	-	30	100→60	-	-
Liver:						
Biliary hyperplasia/periportal inflammation	-	-	-	100	-	-
Skeletal muscle:						
Degeneration/regeneration	200	300	-	-	200	300
Stomach:						
Glandular stomach: ↑ severity erosion	-	300	-	-	-	-
Non-glandular stomach: Hyperkeratosis	-	≥100	-	-	-	300
Non-glandular stomach: Ulceration	-	-	-	100	200	-

* Dose (mg/kg/day) at which finding or changed incidence of finding was observed

The following table shows the incidences of histopathological observations in heterozygous mice with and without iron-supplementation including *p*-cresidine control.

Tissue Findings	MALE							FEMALE						
	GROUPS*							GROUPS						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
EYE														
Atrophy, retina, minimal					1									
Cataract, minimal					2							1		
Cataract, slight					1							4	1	
Cataract, moderate					4							8		
Cataract, marked					1									
Mineralization, lens, slight					1									
Mineralization, cornea	1		1	3										
Necrosis, single cell, lens, minimal							1					1		
KIDNEY														
Loss of normal vacuolation, tubular, epithelium, focal	23	21	16	3			2					1	2	
Vacuolation, tubular, epithelium, diffuse						1						1	2	
Basophilic tubules	10	7	8	8	15	15	14							
LIVER														
Biliary hyperplasia											1		6	2
SKELETAL MUSCLE														
Degeneration, myofiber, focal			2		21									
STOMACH														
Erosion, glandular	7	5	4	5	8	8		6	6	7	6	8	8	8
Hyperkeratosis			2					1	2	4	6	14		1

*: Groups:

Group 1: Control, Iron-supplemented feed

Group 2: Control, Standard feed

Group 3: Male: 20 mg/kg/day, Female: 30 mg/kg/day (Iron-supplemented feed)

Group 4: Male: 100 mg/kg/day, Female: 100 mg/kg/day (Iron-supplemented feed)

Group 5: Male: 200 mg/kg/day, Female: 300 mg/kg/day (Iron-supplemented feed)

Group 6: Male: 30 mg/kg/day, Female: 100→60 mg/kg/day (Standard feed)

Group 7: p-Cresidine 400 mg/kg/day (Standard feed)

The following table shows incidences of histopathological findings in wild type mice.

Tissue Findings	MALE						FEMALE					
	Dose (mg/kg/day)						Dose (mg/kg/day)					
	0	0	200				0	0	300			
EYE												
Cataract, minimal									1			
Cataract, slight									1			
Cataract, moderate									1			
Cataract, marked									1			
KIDNEY												
Loss of normal vacuolation, tubular, epithelium, focal	20	22						1	2			
Vacuolation, tubular, epithelium, diffuse			1									
Basophilic tubules	7	11	9				6	5	8			
SKELETAL MUSCLE												
Degeneration, myofiber, focal			4						2			
Regeneration, myofiber, focal	1	1	10				1	1	3			
STOMACH												
Erosion, glandular	5	2	3				3	3	6			
Hyperkeratosis							3	1	14			
Ulceration, nonglandular			1						1			

Neoplastic: There were no treatment-related neoplastic findings. The tumor incidences are shown in the following table. It is to be mentioned here that the sponsor provided limited historical control data for p53 heterozygous and wild type mice from three studies conducted at this facility during the period of 1999-2003. The sponsor did not perform statistical analysis of the tumor incidences.

MALE							
Organ/Tumor	IRON-SUPPLEMENTED FEED				NON-SUPPLEMENTED FEED		
	0 mg/kg/day (n = 25)	20 mg/kg/day (n = 25)	100 mg/kg/day (n = 25)	200 mg/kg/day (n = 25)	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)	P-Cresidine (400 mg/kg/day) (n = 25)
GROUP	1	3	4	5	2	6	7
Heart							
Lymphoma		1 (4%)					
Stomach							
Lymphoma		1 (4%)					
Duodenum							

Lymphoma		1 (4%)					
Ileum							
Lymphoma		1 (4%)					
Cecum							
Lymphoma							
Pancreas							
Lymphoma		1 (4%)					
Liver							
Lymphoma		2 (8%)					
Transitional cell carcinoma							1 (4%)
Adrenal							
Lymphoma		1 (4%)					
Pheochromocytoma							1 (4%)
Cortical adenoma			1 (4%)		1 (4%)	1 (4%)	
Pituitary							
Lymphoma		2 (4%)					
Epididymides							
Lymphoma		1 (4%)					
Leiomyosarcoma						1 (4%)	
Kidney							
Lymphoma		2 (8%)					
Prostate							
Transitional cell carcinoma							2 (8%)
Urinary Bladder							
Metaplasia, squamous, epithelium, focal, minimal							10 (40%)
Same, slight							6 (24%)
Same, moderate							5 (20%)
Squamous cell carcinoma							1
Transitional cell carcinoma							16 (64%)
Transitional cell papilloma							10 (40%)
Bone Marrow							
Lymphoma		2 (8%)					
Lymph Node-Axillary							
Lymphoma		2 (8%)					
Lymph Node-Bronchial							
Lymphoma		2 (8%)					
Lymph Node-Mandibular							
Lymphoma		2 (8%)					
Lymph Node- Mesenteric							
Lymphoma		2 (8%)					
Thymus							

Lymphoma		2 (8%)				1 (4%)	
Spleen							
Lymphoma		2 (8%)					
Systemic/Any Site							
Lymphoma		2 (8%)				1 (4%) [3.8%]*	
Preputial gland							
Metaplasia, squamous, focal, minimal							1 (4%)
Skin							
Carcinoma							1 (4%)
Bone-Sternum							
Lymphoma		1 (4%)					
Brain							
Lymphoma		1 (4%)					
Lung							
Lymphoma		2 (8%)					
Transitional cell carcinoma							1 (4%)
Trachea							
Lymphoma		1 (4%)					
Eye							
Lymphoma		1 (4%)					
Harderian Gland							
Adenoma							1 (4%)

*: Historical control data from this testing facility from 1999-2003

FEMALE							
Organ/Tissue Finding	IRON-SUPPLEMENTED				NON-SUPPLEMENTED		
	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)	100 mg/kg/day (n = 25)	300 mg/kg/day (n = 25)	0 mg/kg/day (n = 25)	100→60 mg/kg/day (n = 25)	p-Cresidine (400 mg/kg/day) (n = 25)
GROUP	1	3	4	5	2	6	7
Heart							
Lymphoma		1 (4%)					
Stomach							
Lymphoma						1 (4%)	
Pancreas							
Acinar cell adenocarcinoma						1 (4%)	
Liver							
Lymphoma		1 (4%)					

Hepatocellular adenoma							1 (4%)
Pituitary							
Lymphoma		1 (4%)					
Kidney							
Lymphoma		1 (4%)					
Ovary							
Lymphoma		1 (4%)					
Urinary Bladder							
Metaplasia, squamous, epithelium, focal, minimal							7 (28%)
Same, slight							14 (56%)
Transitional cell carcinoma							5 (20%)
Transitional cell papilloma							6 (24%)
Papilloma, squamous cell							2 (8%)
Uterus							
Osteosarcoma				1 (4%)			
Bone Marrow							
Lymphoma		1 (4%)					
Lymph Node							
Lymphoma, bronchial		1 (4%)				1 (4%)	
Lymphoma, pancreatic						1 (4%)	
Thymus							
Lymphoma	1 (4%)	1 (4%)					2 (8%)
Spleen							
Lymphoma		1 (4%)					1 (4%)
Systemic/Any Site							
Lymphoma	1 (4%)	1 (4%)					2 (8%) [5.4%]*
Mammary Gland							
Adenocarcinoma, onset day = 121			1 (4%)				
Skin							
Osteosarcoma, onset day = 172	1 (4%)						
Lung							
Lymphoma	1 (4%)	1 (4%)					1 (4%)

*: Historical control data from this testing facility from 1999-2003

The following table shows the neoplastic findings in Groups 8 and 9 (Standard Feed, Females)

Organ/Tissue	FEMALES (Non-Supplemented Feed)	
	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)
Digestive System		
Lymphoma, salivary gland	1 (4%)	
Hemolymphatic System		
Lymphoma, lymph node, mesenteric	1 (4%)	

The following table shows the neoplastic findings in the wild type animals.

ORGAN/TISSUE FINDINGS	MALES (n = 25)			FEMALES (n = 25)		
	+ Fe	-Fe	+Fe	+ Fe	-Fe	+Fe
Dose (mg/kg/day)	0	0	200	0	0	300
Hepatobiliary System						
Histiocytic sarcoma				1 (4%)		
Genitourinary System						
Endometrial stromal polyp				1 (4%)	1 (4%)	
Hemolymphatic System						
Histiocytic sarcoma				1 (4%)		
Lymphoma, lymph node, axillary				1 (4%)		
Lymphoma, lymph node, bronchial				1 (4%)		
Histiocytic sarcoma, lymph node, bronchial				1 (4%)		
Lymphoma, lymph node, mandibular				1 (4%)		
Lymphoma, lymph node, mediastinal				1 (4%)		
Lymphoma, lymph node, mesenteric				1 (4%)		
Lymphoma, lymph node, renal				1 (4%)		
Lymphoma, lymph node, sublumbar				1 (4%)		
Histiocytic sarcoma, thymus				1 (4%)		
Histiocytic sarcoma, spleen				1 (4%)		
Systemic/Any Site						
Mast cell tumor			1 (4%)			
Lymphoma				1 (4%)		
				[1.8%]*		
Histiocytic sarcoma				1 (4%)		
Respiratory system						
Histiocytic sarcoma, lung				1		

*: Historical control data from this facility from 1999-2003

Toxicokinetics: Based on $AUC_{(0.5-6h)}$ /dose values, there appeared to be no significant gender differences in exposure of p53 heterozygous type mice supplemented with dietary iron. However, in non-supplemented animals, there was an apparent gender difference in drug exposure (females were more exposed than males). At the low dose level, the exposure of p53 heterozygous mice was lower in males and higher in the females when food was not supplemented with iron. Exposure appeared to be over proportional between doses of 20 to 200 mg/kg/day in p53 heterozygous males and between doses of 30 to 300 mg/kg/day in p53 heterozygous females, when feed was supplemented with iron. Female p53 wild type mice were less exposed to ICL670 at the high dose when compared to heterozygous mice. Mean TK parameters are shown in the following table (from page 43 of the study report).

Table 4-8 Mean toxicokinetic parameters of total ICL670 in mouse

	Dose (mg/kg/day)		C_{max} /Dose ($\mu\text{g/mL}$)/(mg/kg/day)		$AUC_{(0.5-6h)}$ /Dose ($\mu\text{g}\cdot\text{hours/mL}$)/(mg/kg/day)	
	Male	Female	Male	Female	Male	Female
Iron supplemented feed						
Group 3 (Low dose group)	20	30	0.655	0.590	1.98	1.90
Group 4 (Mid dose group)	100	100	0.620	0.659	2.03	2.07
Group 5 (High dose group)	200	300	0.910	1.15	3.19	3.66
Group 3w* (High dose group)	200	300	*	0.500	*	1.93
Iron non-supplemented feed						
Group 6 (Low dose group)	30	100→60	0.560	0.907	1.30	3.24
Group 9 (Low dose group)	-	30	-	0.883	-	3.29

*Group 3w (wild type mice). Due to the lack of data, no toxicokinetic analysis was performed for males in this group

Summary Study Findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The dose selection was in accordance with the ExecCAC recommendations (Appendix-3, 4, and 5). The positive control, *p*-cresidine, produced expected tumor responses in the urinary bladder. It is to be mentioned here that the sponsor provided limited historical control data for p53 heterozygous and wild type mice from three studies conducted at this facility during the period of 1999-2003. The sponsor did not conduct statistical analysis of the tumor data.

ICL670 was found to be negative in the Ames assay and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes, positive in micronucleus assay in V79 cells (non-GLP). In the *in vivo* rat (non-supplemented) bone marrow micronucleus test by oral route, ICL 670 was found to be positive at 500 mg/kg dose level. In another oral bone marrow micronucleus test in iron-overloaded rats, ICL670 was shown to be negative at 500 mg/kg dose. At 300 mg/kg dose, ICL 670 was also shown to be negative in the rat bone marrow micronucleus test

irrespective of the iron-overloading of the rats. The appropriateness of the 26-week p53 (+/-) transgenic mouse assay was discussed at the CDER PTCC on August 16, 2001. The Committee considered that the positive genotoxicity results of the micronucleus assay in non-iron-supplemented rats are relevant and recommended that it is appropriate to conduct p53 (+/-) transgenic mouse assay in both iron supplemented and non-iron-supplemented animals, which will help in the interpretation of study results. Overall, the study was designed according to ExecCAC recommendations and appeared to be adequate and acceptable.

Evaluation of tumor findings: There were no test article-related neoplastic findings. The sponsor did not conduct statistical analysis of the tumor data. Based on the results from this study, ICL670 administered orally via gavage for 26 weeks was not carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and at doses up to 300 mg/kg/day for females irrespective of iron supplementation. As expected, the administration of *p*-cresidine caused proliferative changes notably in the urinary bladder of p53 heterozygous mice.

Carcinogenicity Summary: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received *p*-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The doses for the current study were selected based on MTD and as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002 and November 14, 2002, Appendix-3, 4, and 5, respectively).

Test article-related moribundity/mortality occurred in one high dose p53 heterozygous female at 300 mg/kg/day with dietary iron supplementation and one high dose p53 heterozygous female at 100 mg/kg/day without dietary iron supplementation. Nine additional p53 heterozygous mice (four females, supplemented with dietary iron at 300 mg/kg/day and five females, without dietary iron supplementation at 100 mg/kg/day) were also considered test article-related. Test article-related moribundity was also observed in one high-dose wild type male at 200 mg/kg/day with dietary iron supplementation. Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female

mouse at 300 mg/kg/day. There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation. The positive control, *p*-cresidine, produced effects, which included proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%). Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were confined generally to non-supplemented mice.

Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Carcinogenicity Conclusions: Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Recommendations for further analysis: None.

Labeling Recommendations: None

Addendum/Appendix Listing:

Appendix-3: Executive CAC meeting minutes dated August 21, 2001, page: 100

Appendix-4: Executive CAC meeting minutes dated September 19, 2002, page: 105

Appendix-5: Executive CAC meeting minutes dated November 14, 2002, page: 110

Appendix-6: List of neoplastic and non-neoplastic lesions, page: 116

Dose-Ranging Study Report: None

CAC Report: None

Alternative Study Protocols and CAC Report: None

SUMMARY AND EVALUATION:

ICL670 is an orally active iron chelator belonging to a new class of tridentate iron chelators, the N-substituted bis-hydroxyphenyl-triazoles. Novartis has developed EXJADE for the intended indication of the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients. The sponsor has submitted reports of a 2-year oral (gavage) carcinogenicity study with ICL670 (Deferasirox/Exjade®) in Wistar Hannover rats (study no. 017022) and a 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice (study no. 0270117) in NDA 21-882.

In a 2-year oral (gavage) carcinogenicity study (017022) in Wistar Hannover rats, ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). The ICL670 doses were decreased on Day 29 of the study based upon the recommendation received from FDA's Executive CAC (Carcinogenicity Advisory Committee). Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates. In conclusion, based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks does not appear to be carcinogenic at doses up to 100→60 mg/kg/day.

In a 26-week carcinogenicity study (0270117) in p53 transgenic (+/-) mice, ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The doses for the current study were selected based on MTD and as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 19, 2002 and November 14, 2002, Appendix-3, 4, and 5, respectively). Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female mouse at 300 mg/kg/day. There were no treatment-related neoplastic findings either in the p53 heterozygous

mice or wild type mice in the presence or absence of iron supplementation. It is to be mentioned here that the sponsor provided limited historical control data for p53 heterozygous and wild type mice from three studies conducted at this facility during the period of 1999-2003 and did not conduct statistical analysis of tumor incidences. As expected, the positive control, *p*-cresidine, caused proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%). Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were confined generally to non-supplemented mice. Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

RECOMMENDATION: Based on the results of the 2-year carcinogenicity study in rats and 26-week carcinogenicity study in p53 heterozygous mice, ICL670 does not appear to be carcinogenic in rats and p53 heterozygous mice.

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Date

Comments:

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HFD-180/Dr. Choudary

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HFD-160/Dr. Laniyonu

R/D Init. J Choudary: 8/23/05

APPENDIX/ATTACHMENTS

Appendix-1: Exec CAC meeting minutes dated June 5, 2001 for rat carcinogenicity study, page: 65

Appendix-2: List of neoplastic and non-neoplastic lesions for rat carcinogenicity study, page: 69

Appendix-3: Executive CAC meeting minutes dated August 21, 2001 for mouse carcinogenicity study, page: 100

Appendix-4: Executive CAC meeting minutes dated September 19, 2002 for mouse carcinogenicity study, page: 105

Appendix-5: Executive CAC meeting minutes dated November 14, 2002 for mouse carcinogenicity study, page: 110

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APPENDIX-1

Exec CAC Meeting Minutes dated June 5, 2001 for Rat Carcinogenicity Study

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Executive CAC

June 5, 2001

Committee:

Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Ke Zhang, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Ke Zhang

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 58,554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

Background:

ICL-670, an iron chelator, is currently under clinical investigation for transfusion-dependent Sponsor submitted a protocol
for 2-year carcinogenicity study in rats along with the report of a
26-week oral toxicity study in rats in this submission.

Rat Carcinogenicity Dose Selection:

The proposed route of administration is oral gavage. Wistar rats (50/sex/group) will be given ICL-670 at 25, 50, and 100 mg/kg/day. There are two control groups. Animals in control 1 group will receive a standard diet. Animals in control 2 group and the three treatment groups will be fed with an iron supplemented diet (~1680 ppm). Toxicokinetic parameters will be conducted in 10 animals/sex in each group during weeks 4 and 52. The treatment duration is 104 weeks. The following observations will be conducted: clinical signs of toxicity, body weight, food consumption, ophthalmoscopy, clinical pathology including CBC and serum iron, renal parameters, tissue iron analysis (liver and kidney), organ weights, gross and histopathology. The sponsor did not clearly indicate whether the histopathology will be conducted in all animals from all groups.

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The dose selection was based on the results of the 26-week oral toxicity study in rats. In this study, ICL-670 was given to rats by oral gavage at 30, 80, and 180 mg/kg/day. The high dose of 180 mg/kg/day was lethal and exceeded the MTD. ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, ophthalmologic toxicity (cataracts) and increased kidney weights were observed at the low dose tested (30 mg/kg/day). Therefore, the MTD was identified as between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was a no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to the MTD in females. In the 4-week dose ranging studies, the dose of 100 mg/kg/day was lethal. It appears that the high dose of 100 mg/kg/day proposed by sponsor is excessive.

The sponsor stated that a clinically effective dose is expected between 5 and 40 mg/kg. The AUC_{0-24hr} of ICL670 in humans following a single oral dose of 20 mg/kg was 388 $\mu\text{mol.h/l}$ or 144.5 $\mu\text{g.h/ml}$. The AUC_{0-24hr} of ICL670 in rats following an oral dose of 80 mg/kg/day (431 $\mu\text{g.h/ml}$ in males or 314 $\mu\text{g.h/ml}$ in females at week 12) is approximately 2-3 folds of that (144.5 $\mu\text{g.h/ml}$) in humans after 20 mg/kg.

Executive CAC Recommendations and Conclusions:

The Committee did not concur with the sponsor's proposed doses. The Committee recommended doses of 60, 30, and 15 mg/kg/day due to concerns for toxicity at higher doses and a concern that the high dose proposed by the sponsor is above the MTD.

It is recommended that the histopathological examination be conducted in all animals from all groups. If the sponsor plans histopathological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances:

- (a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups,
- (b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group,
- (c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI

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76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,

(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\n
/Division File, HFD-180
/Dr. Choudary, HFD-180
/Dr. Zhang, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

APPENDIX-2

List of Neoplastic and Non-Neoplastic Lesions for Rat Carcinogenicity Study

Appears This Way
On Original

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 DLNCEE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
CARDIOVASCULAR SYSTEM											
HEART	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	10	12	14	11	11	29	29	27	27	24	
Cardiomyopathy.	39	37	36	38	38	21	21	22	22	23	
Hyperplasia, endocardium.				2						1	
Bacteria.								1			
NERVE SHEATH TUMOR (M), endocardium.		1						1		2	
Inflammation, acute.								1			
Fibrosis, pericardium.									1		
AORTA	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	49	50	50	50	50	50	50	50	49	50	
Mineralization.	1								1		
ARTERY		(1)		(1)							
Periarteritis.		1		1							
DIGESTIVE SYSTEM											
SALIVARY GLAND	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	
Within normal limits	48	48	48	49	49	48	49	48	49	48	
ADENOCARCINOMA (M).				1							
Atrophy, acinar.	1										
Eosinophilia, focal.	1	1				1					
Inflammation	1		1		2		1	1			
Metaplasia, squamous.			1								

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
DIGESTIVE SYSTEM											
SALIVARY GLAND	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	
Atrophy, acinar, focal.								1	1	1	
Hemorrhage.											
TRACHEA	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	50	50	50	50	50	50	50	50	50	50	
ESOPHAGUS	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)	
Within normal limits	50	50	50	49	50	50	50	48	48	49	
Inflammation, acute.								2	1		
Perforation.											
Hemorrhage.									1		
STOMACH	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	39	41	37	40	43	44	38	41	39	41	
Congestion, focal.					1						
Cyst, glandular.		1									
Cyst, nonglandular.		1	1				2	2	4	2	
Dilatation, gland, glandular stomach.	1										
Edema, nonglandular.		1	1	1		1	4	2	2		
Erosion, nonglandular.				2	2	2	2	1	1		
Erosion, glandular stomach.		1	1	2	1	1	1	4		1	
Hemorrhage, glandular stomach.				1							
Hyperplasia, mucosa, glandular stomach, focal.						1				1	

Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
ICH570: 104-week oral (gavage) carcinogenicity study in rats
Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
DIGESTIVE SYSTEM											
STOMACH		(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Infiltrate, lymphocyte, glandular stomach, focal.				1	1	1					
Inflammation, nonglandular				1	1	1					
Mineralization.		1	1		1		1				1
Ulcer, nonglandular.					1				1		
Vacuolation, epithelium.			1								
Vacuolation, epithelium, limiting ridge.		8	4	8	4	3	2	2		2	
Inflammatory cell infiltrate, submucosa.							1				
Hyperplasia, focal, epithelium, nonglandular.				1							
Ulcer, glandular.											
LEIOMYOSARCOMA (M).							1		1		1
Inflammation								2			
Hemorrhage, limiting ridge, focal.											1
Hemorrhage, glandular stomach, multifocal.										1	
Inflammation, nonglandular, acute, focal.											1
Hyperplasia, epithelium, nonglandular.										1	
Edema, glandular stomach.					1				1	1	
SQUAMOUS CELL CARCINOMA (M).											1

(M) Malignant tumor
Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 Page : 4
 PLACES 2400 VI.000

DOSE/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day
DIGESTIVE SYSTEM											
STOMACH	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
SARCOMA (M)										1	
DUODENUM	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	48	50	48	47	50	50	48	50	49	50	
Dilatation, segmental.				1							
Diverticulum.	1		1	1			1				
Inflammation	1		1	1					1		
Mineralization.			1								
Ulceration.			1								
Mineralization, focal.				1							
Infiltrate, eosinophil.							1				
JEJUNUM	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	50	47	50	50	49	50	49	49	49	50	
Dilatation.		1			1			1			
Erosion.		1									
Fibrosis, focal.		1									
Inflammation					1			1	1		
Bacteria, serosa, acute.									1		
Focal.											
Ulceration.								1			
Parieteritis.							1				
ILEUM	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	50	49	50	49	50	50	49	50	50	50	

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICD670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
DIGESTIVE SYSTEM											
ILEUM											
Dilatation.	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
		1						1			
CECUM											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Dilatation.	50	49	48	50	48	49	48	50	47	50	
Diverticula.		1	1						1		
Erosion.					1		1				
Inflammation				1	2				1		
LEIOMYOSARCOMA (M).									1		
Infiltrate, eosinophil.							1		1		
Aggregation, macrophage, increased.							1		1		
COLON											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Dilatation.	50	49	47	49	50	50	50	49	50	49	
Infiltrate, eosinophil.		1	1							1	
Intussusception.			1								
Pigment, lamina propria.			1								
LEIOMYOSARCOMA (M).				1							
RECTUM											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Edema.	50	50	49	50	50	50	50	49	50	49	
SARCOMA (M)			1					1		1	

(M) Malignant tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25-35 mg/kg/ day	50-30 mg/kg/ day	100-30 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25-35 mg/kg/ day	50-30 mg/kg/ day	100-30 mg/kg/ day
DIGESTIVE SYSTEM											
MESENTERY											
Accessory spleen.		(1)		(2)	(3)			(2)	(1)		(1)
Fat necrosis.					1			1			1
Hemorrhage, focal.					1						1
PANCREAS											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Alteration, basophilic, focal.	37	38	37	36	37	38	33	34	40	43	
Atrophy, acinar.	1	1		1	2			1			
Dilatation, duct.	5	4	7	7	4	5	11	6	5	3	
Edema.	1	2	2	3	1		1	1	1		
Fibrosis, perivascular.						1					
Hyperplasia, islet cell, focal.	1		1	1		1	1	2			
Inflammation	3	6	1	4	7	4	4	5	4	4	
ISLET CELL ADENOMA [B].	1		2		1	1					
ISLET CELL CARCINOMA [M].	1		1	3	1						
Periarteritis.						2		1			
Hemorrhage.									1		

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 VLACS 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
HEPATOBIILIARY SYSTEM											
BILE DUCT - INTRAHEPATIC Dilatation.	(2) 2	(3) 3	(2) 2	(4) 4	(2) 2	(3) 3	(3) 3		(2) 2	(1) 1	
LIVER	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	10	10	6	8	14	7	3	6	11	12	
Adiectasis.	1	1	2	2	1	2	2	3	1	1	
Cyst, biliary.	1	2		2	3	5	4	2	1	5	
Degeneration, cystic.				3	1				1		
Dilatation, bile duct, focal.				2							
Dilatation, sinusoid, focal.	15	7	7	8	8	7	9	4	3	3	
Fibrosis			1			1				1	
Focus, acidophilic.	14	18	15	16	12	5	7	11	4	5	
Focus, basophilic.	1	1		1	1		2	1	1		
Focus, basophilic, tigroid.	21	16	18	14	16	31	33	24	22	26	
Focus, clear cell.	1		1			1					
HEPATOCELLULAR ADENOMA [B].	1		1	1	2	2	2	3			
HEPATOCELLULAR CARCINOMA [N].		1	1	2	2	1	1	1			
Hernia, diaphragmatic.		1	1					1			
Hyperplasia, bile duct.	7	5	10	8	7	15	11	10	11	11	
Hyperplasia, hepatocellular, focal.			1								
Infiltrate, lymphocyte, focal.		2			1	1	1	1		2	
Inflammation	5	3	8	8	7	3	3	5	8	4	
Mineralization, focal.				1					1		

[B] Benign tumor

[N] Malignant tumor

Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 TCE670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 BLACRE 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/day	0 ng/kg/day	25-35 ng/kg/day	50-30 ng/kg/day	100-360 ng/kg/day	0 ng/kg/day	0 ng/kg/day	25-35 ng/kg/day	50-30 ng/kg/day	100-360 ng/kg/day
HEPATOBIILIARY SYSTEM											
LIVER	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Mitotic figures, increased.						1				2	
Necrosis, focal.	1	1	1					1	1		
Necrosis, centrilobular.			1							1	
Necrosis, single cell, focal.		1								1	
Pigment, hepatocellular, Kupffer cell.	1	2				1	1	3	3	1	
Tension focus.	1	1	1	2	2	2	3	3	2	1	
Vacuolation.	3	2	5	2	2	3	4	1	1	2	
Vacuolation, focal.	4	1	6	4	4	1	1	1	4	2	
Vacuolation, centrilobular.	1	1	2	1				1	2	1	
Extramedullary hematopoiesis.	2	3	2	3	2	3	3	3	2	2	
Necrosis.								1			
Fibrosis, periportal.									1		
Deformity, focal.								1			
CHOLANGIOMA [B]								1			
Pigment, macrophage.			1				1				
ENDOCRINE SYSTEM											
ADRENAL	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	27	28	25	31	29	10	5	10	17	12	
Angiectasis, cortex.	3			1	5	24	32	28	27	32	
Angiectasis, medulla.							1				
Atrophy, cortex.					1			2			
Cyst.		1								1	

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL570: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACEB 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
ENDOCRINE SYSTEM											
ADRENAL		(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Hypertrophy, cortex, focal.				1	2		1		3	1	1
Hypertrophy, cortex, cystic, focal.	13	13	17	10	9	10	15	14	8	11	
Infiltrate, lymphocyte, focal.					1						
PHEOCHROMOCYTOMA [B].	3	2	1	1				1			
Vacuolation, cortex, focal.	3	6	5	3	4	1					
CORTICAL ADENOMA [B].				1	2	2	1		1	1	
Hyperplasia, focal, cortex.	1		1		1						1
Hyperplasia, focal, medulla.											
PHEOCHROMOCYTOMA [M].							1				
Periarteritis.							1				
Necrosis, cortex.							1				
Hypertrophy, cortex.							1	1	2	2	
Hemorrhage.							1	1	3		1
HEMANGIOBLASTOMA [M].							1				
Fibrosis, cortex.							2		1		
Extramammary metaplasia, increased.	3	1		2	1		5	2	2	3	
Infiltrate, lymphocyte.		1									
PANCREAS		(45)	(50)	(48)	(49)	(48)	(46)	(47)	(47)	(47)	(48)
Within normal limits.		43	42	40	44	43	45	43	45	47	47
Hyperplasia.		1		1							

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICG670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACRE 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
ENDOCRINE SYSTEM											
PARATHYROID	(49)	(50)	(48)	(49)	(48)	(46)	(47)	(47)	(47)	(48)	
Hyperplasia, focal.	4	7	5	4	5		3			1	
ADENOMA [B].	2	1	1	1			1	2			
PITUITARY	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	19	23	17	21	16	15	12	17	14	13	
ADENOMA [B], pars distalis.	17	15	19	15	20	28	29	22	29	28	
ADENOMA [B], pars intermedia.	1	1	2			1	1				
CARCINOMA [M], pars distalis.											
Cyst.	10	4	5	4	1	7	2	1	3	2	
Hemorrhage						1					
Hyperplasia, focal, pars distalis.	8	8	11	9	6	6	8	8	6	7	
Hyperplasia, focal, pars intermedia.	1				1		1				
THYROID	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	
Within normal limits	27	26	34	25	24	37	21	30	31	26	
C-CELL ADENOMA [B].	3	3	7	8	9	5	13	6	7	5	
C-CELL CARCINOMA [M].		1		2				1			
Cyst, follicular.	2			2	1	1		1			
Cyst, ultimobranchial.		1			1			1		3	
Ectopic thymus.					1						
Hyperplasia, c-cell, diffuse.	3	3		6	2	2	5	5	5	6	
FOLLICULAR ADENOMA [B].	4	5	4	2		3	1	1	1	1	

[B] Benign tumor
 [M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
ENDOCRINE SYSTEM											
THYROID	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	
Hyperplasia, focal, c-cell.	8	4	2	4	12	5	9	8	7	8	
Hyperplasia, focal, follicular.	5	5		3	3	1	2	2		2	
Inflammation									1	1	
FOLLICULAR ADENOCARCINOMA [M].	1									1	
GENITOURINARY SYSTEM											
CERVIX						(50)	(50)	(50)	(50)	(50)	
Within normal limits						45	47	48	50	49	
Cyst.							1				
LEIOMYOSARCOMA [M].							1				
Hypertrophy.						3				1	
Hyperplasia, epithelium.						1					
Aggregation, granular cell.						1					
PULP [B].							1				
EPIDIDYMIDES	(50)	(50)	(50)	(50)	(50)						
Within normal limits	48	42	43	42	45						
Aspermia.	2	7	6	5	5						
Edema.				1							
Fibrosis.		1									
Granuloma, sperm.		2	1	2	1						

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day
GENITOURINARY SYSTEM										
EPIPIDYMIDES										
Hamartoma, vascular.	(50) 1	(50) 1	(50) 1	(50) 1	(50) 1					
Inflammation		1	1	1						
MESOTHELIONA [M].										
Inflammation, chronic, focal.			2	2	1					
KIDNEY										
Within normal limits	(50) 14	(50) 15	(50) 13	(50) 18	(50) 28	(50) 8	(50) 8	(50) 7	(50) 6	(50) 12
AUSEKOMA [B].					1					
Basophilic tubules.	3		1	3	3	1	1			3
Calculi.	2		1	1	1	1	3			1
Cast, proteinaceous.				1	3	2		4	3	4
Chronic progressive nephropathy.	29	27	31	22	11	7	13	10	8	9
Cyst.	2	1	2	2	1		1	2		1
Dilatation, tubular.		4		1	1	1		1	3	2
Hemorrhage.		1								
Hyaline droplets.	1		1	1				1	1	
Hydronephrosis.		5		1	3			1	1	
Hyperplasia, epithelium, pelvic.					1					
Hyperplasia, epithelium, pelvic, focal.	1		1			2	3	6	5	2
Infarct, chronic.	1									
Inflammation	1	1	5	3	4	1		3	4	2

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day
GENITOURINARY SYSTEM										
KIDNEY		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
LIPIDURONIA [M]				1	1					
Mineralization, focal		2	5	1	1		23	18	19	22
Mineralization, epithelial, pulvis, focal		9	2	1	2	2	34	23	24	23
Necrosis, medulla			1							
Necrosis, papillary		1				1			1	1
Necrosis, tubular				1	2	2	4	4	4	4
Periarteritis				1	1					
Pigment, tubular				1	1		2	2	1	3
Pyelonephritis		1							1	2
Hypertrophy, focal, tubular			1							1
Bacteria										
Fibrosis							1			1
TRANSITIONAL CELL PAPILLOMA [B]		1								
TRANSITIONAL CELL CARCINOMA [M]					1					
OVARY							(50)	(50)	(50)	(50)
Within normal limits							39	43	41	41
Cyst							9	4	6	7
Cyst, hemorrhagic									1	
ADENOCARCINOMA [M]										1
THECAL CELL TUMOR [M]										1

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 14-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day
GENITOURINARY SYSTEM											
OVARY											
THECAL CELL TUMOR [B].						(50)	(50)	(50)	(50)	(50)	
LUTEAL CELL TUMOR [B].							1			1	
Hemorrhage.						1	1		1	1	
Hyperplasia, tubulostromal.									1	1	
Atrophy.									1	1	
PROSTATE	(50)	(50)	(50)	(50)	(50)						
Within normal limits	33	31	25	34	35						
ADENOCARCINOMA [M].		1		1							
Inflammation	14	16	22	14	13						
Secretion reduced.		1	1		1						
Hyperplasia, focal.	4	2	1	2	3						
ADENOMA [B].		1									
SEMINAL VESICLE	(50)	(50)	(50)	(50)	(50)						
Within normal limits	48	43	42	45	44						
ADENOCARCINOMA [M].	1										
Dilatation.		1									
Inflammation	1		2	1	3						
Secretion reduced.		5	5	4	5						
TESTIS	(50)	(50)	(50)	(50)	(50)						
Within normal limits	41	37	37	38	42						
Atrophy, seminiferous tubule.	7	9	9	10	8						

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACEB 2000 VI.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->25 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->25 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
GENITOURINARY SYSTEM											
TESTIS	(50)	(50)	(50)	(50)	(50)						
Edema.		1	3	1	3						
Granuloma, sperm.		1									
INTERSTITIAL CELL TUMOR [B].		3	1	1							
Mineralization, focal.				1							
Inspissation, sperm.	1	1			2						
Hyperplasia, focal, interstitial cell.				1							
URETER		(1)			(1)			(1)	(1)		
Dilatation.		1			1				1	1	
Inflammation, acute.								1	1		
URINARY BLADDER	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	
Within normal limits	47	47	49	49	47	48	50	48	47	49	
Calculi.	1				1				1		
Dilatation.	1	2		1	1				2		
Hemorrhage.		1				1			1		
Hyperplasia, transitional cell.	1				2			1	1	1	
Inflammation											
Edema.	2				2	1			1	1	
						1					
UTERUS						(50)	(50)	(50)	(50)	(50)	
Within normal limits						36	33	32	35	37	

[B] Benign tumor

Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25-35 mg/kg/ day	50-30 mg/kg/ day	100-30 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25-35 mg/kg/ day	50-30 mg/kg/ day	100-30 mg/kg/ day
GENITOURINARY SYSTEM											
UTERUS											
ADENOCARCINOMA [N].						(50)	(50)	(50)	(50)	(50)	
CHORIOCARCINOMA [N].							2	2	1	2	
Cyst.						7	7	8	4	6	
Dilatation.						3	1	1	2	1	
ENDOMETRIAL STROMAL POLYP [B].						3	5	5	7	3	
ENDOMETRIAL STROMAL SARCOMA [N].						1	1	2			
Inflammation.						1	1		1		
Thrombosis.							1				
Necrosis.							1				
Hyperplasia, endometrial.						2	1	1	2		
Hemorrhage.						1	1				
HEMANGIOENDOTHELIOMA [N].						1					
Adenomyosis.							1				
VAGINA											
Within normal limits						(50)	(50)	(50)	(50)	(50)	
Aggregation, granular cell.						44	38	43	47	49	
GRANULAR CELL TUMOR [B].						2	6	2	2	1	
POLYP [B].						2	2	2			
Inflammation							2	1	2		
Prolapse.							1		1		
LEIOMYOSARCOMA [N].						1					

[B] Benign tumor

[N] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
GENITOURINARY SYSTEM											
VAS DEFERENS											
Granuloma, sperm.		(1) 1									
HEMOLYMPHATIC SYSTEM											
BONE MARROW	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	43	40	46	44	46	44	41	45	41	45	
Granulopoiesis, increased.	3	4	1	3	3	1	3	3	4	4	
Neutropoiesis, increased.	2	4		1		3	5		3		
Neutropoiesis, decreased.		1		1	1		1	1	2	1	
Hyperplasia, lymphoid, focal.											
Fibrosis.	2	1	2	1		1		1			
LYMPH NODE	(11)	(11)		(11)							
Ectasia, sinus.		1		1							
Hemorrhage, mediastinal.	1										
LYMPH NODE - BRONCHIAL	(46)	(47)	(47)	(47)	(48)	(48)	(48)	(45)	(46)	(46)	
Within normal limits	38	37	38	40	40	44	45	39	43	44	
Accumulation, erythrocyte.	1	1		2	2		2	2		1	
Ectasia, sinus.											
Infiltrate, neutrophil.	5	6	8	5	6		1	1	1	1	
Pigment, macrophage.						1					
Bacteria.									1		
Aggregation, macrophage, increased.	1	2	1		2	1		1			

Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 BLACKE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
HEMOLYMPHATIC SYSTEM											
LYMPH NODE - INTESTINAL											
Ectasia, sinus.			(1) 1								
LYMPH NODE - MANDIBULAR	(50)	(50)	(50)	(50)	(49)	(49)	(50)	(50)	(50)	(49)	
Within normal limits	44	41	42	46	45	48	44	45	44	43	
Ectasia, sinus.	3	9	8	4	2		4	2	2	3	
Hemorrhage.	1										
Hyperplasia, lymphoid.	1		1				1	1	1	1	
Hyperplasia, plasma cell.	2				2		2	1		2	
Depletion, lymphoid.								1			
Accumulation, erythrocyte.			1						2		
LYMPH NODE - MEDIASTINAL	(4)	(7)	(6)	(7)	(2)	(5)	(3)	(2)	(3)		
Accumulation, erythrocyte.	2	2	1	3	1	3	1	1	3		
Ectasia, sinus.	2	3	4	6	1		1	1			
HEMANGIOENDOTHELIOMA (M)		1									
Infiltrate, neutrophil.						1					
Inflammation, acute.								1			
Hyperplasia, plasma cell.									1		
LYMPH NODE - MESENTERIC	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)		
Within normal limits	35	34	30	32	33	20	20	24	21	16	
Accumulation, erythrocyte.			4	3			2				
Ectasia, sinus.	2	9	6	3	3		4	1	3	3	
Fibrosis.				1			1				
HEMANGIOENDOTHELIOMA (M)				2			1	1			

[M] Malignant tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral [gavage] carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day	100->60 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->15 mg/kg/ day	50->30 mg/kg/ day
HEMOLYMPHATIC SYSTEM										
LYMPH NODE - MESENTERIC		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Histiocytosis, sinus.			1	1						
Hyperplasia, lymphoid.			2			1		2		
Hyperplasia, plasma cell.									1	1
Pigment, macrophage.						1				
Aggregation, macrophage, increased.	13	5	10	10	13	28	23	23	25	32
LYMPH NODE - PANCREATIC		(1)	(1)	(2)	(1)			(1)		
Accumulation, erythrocyte.			1	1						
Ectasia, sinus.	1	1	1	1						
Fibrosis.										
Pigment, macrophage.								1		
LYMPH NODE - POPLITEAL			(1)							
Ectasia, sinus.			1							
LYMPH NODE - RENAL			(2)	(1)						
Within normal limits			1							
Ectasia, sinus.			1	1						
Histiocytosis, sinus.			1							
LYMPH NODE - SUBLINGUAL		(1)	(5)		(1)	(5)	(1)	(1)	(3)	(1)
Ectasia, sinus.		1	4		1	4		1		
HEMANGIOANGIOSARCOMA (H)										
Hemorrhage.					1					

[N] Malignant tumor

Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACKE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->15 ng/kg/ day	50->30 ng/kg/ day	100->60 ng/kg/ day
HEMOLYMPHATIC SYSTEM											
LYMPH NODE - SUBLINGUAL											
Mitoticcytosis, sinus.		(1)	(5)		(1)	(5)	(1)	(1)	(1)	(3)	(2)
Hyperplasia, lymphoid.			1							2	1
Hyperplasia, plasma cell.			1							1	
Necrosis, focal.											
THYMUS		(49)	(50)	(49)	(49)	(50)	(50)	(49)	(49)	(50)	(50)
Within normal limits		43	40	39	39	41	21	25	21	29	24
Atrophy, lymphoid.						1		2	1	1	
Cyst.		3	5	8	6	6	24	21	20	15	19
Hemorrhage.				1	1	1				1	
Hyperplasia, epithelial.				1				1			
THYMOMA [B].		2	1		1	3	2	4	5	5	6
THYMOMA [M].			1					1		1	1
Hyperplasia, focal.			1	1	2		3		2		1
Hemorrhage, focal.							1				
Hyperplasia, lymphoid.									1		
Ectopic parathyroid.			1								
SPLEEN		(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Within normal limits		46	42	43	43	46	39	33	39	35	38
Depletion, lymphoid.					1	1			1	1	
HEMANGIOSARCOMA [M].					1						
Pigment.			1	2		1				2	

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
ICL670: 104-week oral (gavage) carcinogenicity study in rats
Study No. 017922

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->25 mg/kg/day	50->30 mg/kg/day	100->50 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->25 mg/kg/day	50->30 mg/kg/day	100->50 mg/kg/day
HEMOLYMPHATIC SYSTEM											
SPLEEN		(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(49)	
Extramedullary hematopoiesis, increased.		4	6	2	6	2	10	17	9	8	
Periarteritis.							1				
Necrosis, focal.										1	
Hyperplasia, lymphoid.										2	
Hematomas.									1		
Congestion, focal.									1	1	
Accessory spleen.									1		
Cyst, capsule.									1		
Hyperplasia, histiocytic, focal.				1					1		
SYSTEMIC/AMY SITE		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits		48	49	48	50	50	49	50	47	49	
HISTIOCYTIC SARCOMA (M).		2		1					3		
LYMPHOMA (M).			1				1			1	
INTEGUMENTARY SYSTEM											
CLITORAL GLAND							(50)	(49)	(48)	(48)	
Within normal limits							27	22	28	29	
Inflammation.							23	27	20	17	
Dilatation.									1		
Hyperplasia, focal.									1		
SQUAMOUS CELL CARCINOMA (M).										1	

(M) Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
INTEGUMENTARY SYSTEM											
MAMMARY GLAND		(48)	(47)	(50)	(46)	(47)	(50)	(50)	(50)	(50)	(50)
Within normal limits		47	44	49	44	46	30	26	29	32	30
ADENOCARCINOMA [N]							6	3	2	3	1
ADENOMA [B]							1	1		1	1
FIBROADENOMA [B]							4	12	8	3	8
FIBROMA [B]							1	1			
Galactoceles			1	1	2		5	4	5	6	2
Hyperplasia			1			1	8	13	8	10	11
Inflammation										1	
Hyperplasia, with atypia							2		1	1	3
ADENGLIOMA [B]							1				
PREPUTIAL GLAND		(50)	(49)	(49)	(49)	(50)					
Within normal limits		18	19	18	24	24					
Dilatation						1					
Hemorrhage, chronic				1							
Inflammation		32	30	30	25	25					
Mineralization, focal					1						
SKIN		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits		39	33	41	41	40	39	44	40	44	42
Atrophy, follicular		1	6	3	2	2	4	4	7	4	4
BASEAL CELL TUMOR [B]					2	1		1			1
Cyst, inclusion			2	1	2	1			1		
Debris, surface, focal			1		2	1	1				

[B] Benign tumor
 [N] Malignant tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICLE70: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
INTEGUMENTARY SYSTEM											
SKIN		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
FIBROMA [B].		1	2			1					1
FIBROSARCOMA [M].						1					1
Fibrosis.			1		1				1		
HEMANGIOENDOTHELIOMA [M].					1						
Hemorrhage.					1						
Hyperplasia, sebaceous gland, focal.				1						1	
Inflammation		2	4	2	1	3	3		2		
KERATOCYSTITIS [B].		1	5	2	2	1				1	
LIPOMA [B].		1	1						1		
PAPILLOMA [B].		2	1	1			1			1	
Pigment, focal.				1							
Ulceration.		1		1		2	2		1		
NERVE SHEATH TUMOR [M], cystic.			1				1				
SEBACEOUS ADENOMA [B].		1									
SEBACEOUS CARCINOMA [M].											1
BASAL CELL CARCINOMA [M].		1					1	1			
Actinosis.						1	2		3		
NERVE SHEATH TUMOR [M].		1				1					
LEIOMYOSARCOMA [M].							1				

[B] Benign tumor
 [M] Malignant tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-35 mg/kg/day	50-30 mg/kg/day	100-30 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-35 mg/kg/day	50-30 mg/kg/day	100-30 mg/kg/day
MUSCULOSKELETAL SYSTEM											
BONE			(1)	(1)	(1)	(1)	(1)		(1)		
Hyperostosis.					1	1	1				
OSTEOSARCOMA (M).										1	
Bacteria, focal.										1	
Inflammation.										1	
Exostosis.										1	
BONE - FEMUR/TIBIA	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	
Within normal limits.	49	50	50	50	49	49	50	50	50	49	
Chondrodystrophy, joint.					1						
BONE - STERNUM	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	
Within normal limits.	49	49	50	50	50	50	50	49	50	50	
Hyperostosis.	1										
Degeneration, chondromucinous, focal.		1									
DIAPHRAGM						(1)	(1)	(1)	(2)		
Bacteria.									1		
Inflammation.						1	1	1	1		
Fibrosis, focal.								1			
MUSCLE	(49)	(50)	(49)	(50)	(50)	(49)	(50)	(50)	(49)	(50)	
Within normal limits.	46	48	49	45	49	47	48	48	48	49	
Atrophy.	1	1		1	1	1	1		1	1	
HEMANGIOCARCOMA (M).										1	

(M) Malignant tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICLE79: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACE 2000 VI.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
MUSCULOSKELETAL SYSTEM											
MUSCLE											
Hemorrhage, focal.	(45)	(50)	(48)	(50)	(50)	(45)	(50)	(50)	(42)	(50)	
Inflammation	1	1					1	1			
Necrosis	1										
Pigment, focal.							1	1			
HEPATOMYOSARCOMA [M].								1			
Bacteria								1			
GRANULAR CELL TUMOR [B].						1					
Foreign body present.								1			
Thrombus								1			
FIBROSARCOMA [M].				1							
NERVOUS SYSTEM											
BRAIN											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Compression	44	41	36	43	42	39	39	40	37	38	
Edema	3	7	11	5	6	9	10	8	10	10	
Hemorrhage			1								
GRANULAR CELL TUMOR [B].			1							1	
Hydrocephalus			1								
Mineralization, focal.	3	4	8	3	1	5	6	5	10	7	
Mineralization, vascular.		1		1			1				
Meninges											
OLIGODENDROGLIOMA [M].	1			1					1		

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 TCL67D: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->50 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day
NERVOUS SYSTEM										
BRAIN		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
ASTROCYTOMA [M]						1				1
GRANULAR CELL TUMOR [M]				2		1	2	2		1
NERVE - SCIATIC		(49)	(50)	(49)	(50)	(49)	(50)	(50)	(49)	(50)
Within normal limits		48	48	48	50	48	48	50	48	50
Degeneration		1	2			1			1	
SPINAL CORD		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits		17	14	19	19	18	25	23	29	27
Compression						1				
Degeneration, white matter		7	5	5	8	2	8	10	4	7
Degeneration, spinal nerve		33	34	31	29	31	25	24	21	23
RESPIRATORY SYSTEM										
LUNG		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Within normal limits		29	30	40	27	26	35	34	33	29
Accumulation, macrophage, foamy		15	16	9	14	22	9	14	7	11
MEMBRAN [B], bronchioalveolar						1		1		1
Cystic lesion		2		1	1					2
Eosin		1								

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACKE 2000 W1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-30 ng/kg/ day	50-30 ng/kg/ day	100-60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-30 ng/kg/ day	50-30 ng/kg/ day	100-60 ng/kg/ day
RESPIRATORY SYSTEM											
LUNG	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)	
Foreign material present, bronchial.					1						
Hemorrhage.	4	1	1	3			1	1	1	1	
Infiltrate, granulocyte, perivascular.	1	2			1						
Infiltrate, lymphocyte, multifocal.			1								
Inflammation	4	1	3	3	1	3	4	2	3	3	
Myopathy, vein.	1										
Bacteria.								1			
Hyperplasia, focal, bronchiolalveolar.		1			1	2			1	2	
Pigment, macrophage.				1		1				1	
NASAL CAVITY									(1)		
Accumulation, blood, lumen.									1		
TRACHEA	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)	
Within normal limits	50	50	50	49	50	50	50	50	48	49	
Inflammation				1							
SOFT TISSUE											
FAT	(2)	(2)	(1)		(2)	(4)	(6)	(5)	(6)	(5)	
Fat necrosis.		2	1		1	4	6	4	6	5	
Hemorrhage.					1						

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25->125 ng/kg/ day	50->300 ng/kg/ day	100->600 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25->125 ng/kg/ day	50->300 ng/kg/ day	100->600 ng/kg/ day
SOFT TISSUE											
FAT - BECKW Within normal limits		(1) 1									
FAT - PERIOVARIAN Hamartoma.							(1) 1				
SPECIAL SENSE ORGANS											
EAR Within normal limits Chondrodysplasia, pinna.				(1) 1	(1) 1				(1) 1		
EYE Within normal limits	(50) 43	(50) 42	(50) 47	(50) 41	(50) 43	(50) 42	(50) 41	(50) 42	(50) 45	(49) 44	
Absent.			1								
Atrophy, retina.	6	5	2	7	5	7	7	6	4	4	
Cataract.		2									
Cyst, retrobulbar.							1				
Hemorrhage					1	1					
Hyalinization, iris/ciliary body.		2		1	1			1			
Inflammation					1	1			1	1	
Mineralization, cornea	1			1			2			1	
No abnormality detected.		1									
Synechia		1									
Hyperplasia, epithelium, cornea.										1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/day	0 ng/kg/day	25->15 ng/kg/day	50->30 ng/kg/day	100->60 ng/kg/day	0 mg/kg/day	0 ng/kg/day	25->15 ng/kg/day	50->30 ng/kg/day
SPECIAL SENSE ORGANS										
EYE		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(48)
Fluid, proteinaceous, anterior chamber.									1	
Dilatation.								1		1
Exophthalmos.										
PAROTID GLAND		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Within normal limits		40	33	33	33	34	33	35	30	31
Atrophy, acinar.		1	8	6	4	2	12	8	13	13
Inflammation		9	14	12	15	14	11	9	11	9
Cyst.						1				
Hyperplasia, focal.						1				
LACRIMAL GLAND		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits		22	19	13	17	20	45	41	46	41
Inflammation		14	18	18	9	10	4	4	2	6
Metaplasia.		26	26	34	30	27	2	7	2	4
Atrophy, focal.			1				1		2	
TYMBAL'S GLAND			(2)							(1)
CARCINOMA (N).			2							1

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
ICL670: 104-week oral (gavage) carcinogenicity study in rats
Study No. 017022

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PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES											
ABDOMINAL CAVITY Inflammation, wall, acute. Fat necrosis.							(1) 1				(1) 1
FORELIMB No abnormality detected. nail.				(2) 2		(1) 1					
HINDLIMB Within normal limits	(21)	(15)	(18)	(12)	(21)	(10)	(3)	(9)	(5)	(9)	
Edema.	1		1		1						
Exostosis.	1			1							
HEMANGIOsarcoma [M].			1								
Inflammation	1		1	1	1						
Inflammation, nail, acute. No abnormality detected. nail.	10	6	9	8	6	9		2	1	4	
Pododermatitis. No abnormality detected.	15	12	9	8	16	3	3	7	3 1	5 1	
MEDIASTINUM NERVE SHEATH TUMOR [M]. cystic. Bacteria. Inflammation, acute.				(1) 1		(1) 1		(2) 2			

[M] Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
ICL670: 104-week oral (gavage) carcinogenicity study in rats
Study No. 017022

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES											
MEDIASTINUM Foreign body present.					(1)		(1)		(2) 1		
TAIL CHORDOMA [B].		(1) 1									

[B] Benign tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-3

Executive Committee Meeting Minutes Dated August 21, 2001 for Mouse Carcinogenicity Study

Executive CAC
August 7, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Charles Resnick, Ph.D., HFD-110, Alternate Member
Karen DavisBruno, Ph.D., HFD-510, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 26-week carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL 670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias. This drug is currently under Phase I clinical trial. The sponsor has submitted a protocol and dose selection for the 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 30, 100, 300, and 600 mg/kg/day doses.

P53 Mouse Carcinogenicity Dose Selection:

The sponsor has proposed to conduct a 26-week oral (gavage) carcinogenicity study in p53 (+/-) transgenic mice at 30, 100, 300, and 600 mg/kg/day doses. The sponsor's dose selection was based on the results of the previous 4-week oral dose range-finding study in C57BL/6 mice with iron-supplemented diet. In the 4-week oral dose ranging study, the animals were orally administered ICL 670 at 10, 30, 100, and 200 mg/kg/day. There was no mortality. At Week 4, the mean body weight gain in males was reduced, but not in a dose-related manner, at all dose levels (21.8%, 39.2%, 30.5%, and 60.9% reduction at 10, 30, 100, and 200 mg/kg/day, respectively) when compared to body weight gain in controls. The body weight gain in females at Week 4 was also reduced (21.8%, 21.8%, 26.1%, and 17.4% at 10, 30, 100, and 200 mg/kg/day, respectively) compared to control but not in a dose-related manner. The body weight gain data appeared to be erratic, exaggerated (0.9 to 1.9 g weight gain compared to 2.3 g in control) and did not follow any dose-related pattern. These data were not considered reliable to use as the basis of dose selection for the proposed carcinogenicity study for females. The 100 mg/kg/day was identified as the NOEL. At 200 mg/kg/day, there was decreased glycogen

content in the liver of all treated males. Treatment-related histopathological changes were observed in the stomach (focal alteration in the glandular stomach characterized by inflammation, hypertrophy of the chief cells, atrophy of the parietal cells) of males and females at all dose levels in a low incidence; however, the changes were not dose-related.

ICL 670 was found to be negative in the Ames assay and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. The sponsor conducted three in vivo rat bone marrow micronucleus tests by oral route. In the first experiment in non-iron-supplemented rats, ICL 670 showed a marginal but statistically significant increase ($0.14 \pm 0.05\%$, $0.22 \pm 0.11\%$, and $0.27 \pm 0.11\%$ at 50, 158, and 500 mg/kg, respectively, vs. $0.14 \pm 0.06\%$ in control) in the number of micronucleated polychromatic erythrocytes (MPE) at 500 mg/kg at 48 h, which was accompanied by mortality and severe bone marrow toxicity. However, in this study, the 24 h time point was not examined. In the second experiment, the number of MPEs was examined in non-iron-supplemented rats at Day 1, 2, 3, 6, and 10 after the treatment at 500 mg/kg. In this study, the number of MPEs was significantly increased ($0.35 \pm 0.11\%$, $0.36 \pm 0.07\%$, $0.44 \pm 0.27\%$, and $0.76 \pm 0.32\%$ at Day 1, 2, 3, and 6, respectively, vs. $0.13 \pm 0.03\%$ in control) at all time points except at Day 10 ($0.15 \pm 0.03\%$). The third experiment was a direct comparison between rats with or without iron-supplemented diet. ICL 670 was found to be negative at 500 mg/kg in both iron-supplemented ($0.20 \pm 0.09\%$, $0.12 \pm 0.04\%$ at 300 and 500 mg/kg, respectively, vs. $0.17 \pm 0.02\%$ in control) and non-iron-supplemented ($0.20 \pm 0.09\%$, $0.23 \pm 0.06\%$ at 300 and 500 mg/kg, respectively, vs. $0.16 \pm 0.05\%$ in control) rats at 48 h time point. In this experiment also, the sponsor did not study the 24 h time point. ICL 670 was found to be positive in non-iron-supplemented rats at 500 mg/kg at 168 h time point ($0.19 \pm 0.12\%$ and $0.58 \pm 0.04\%$ at 300 and 500 mg/kg, respectively, vs. $0.14 \pm 0.04\%$ in control). It is to be mentioned here that similar increase in the number of MPEs was also observed in the previous experiment in non-iron-supplemented rats, where ICL 670 increased the number of MPEs on Day 6 ($0.76 \pm 0.32\%$ vs. $0.13 \pm 0.03\%$ in control). In addition, in a micronucleus assay using hepatocytes from partially hepatectomized rat, ICL 670 was shown to be negative at 250 and 500 mg/kg at 72 and 96 h sampling time.

There was a discussion about the appropriateness of the p53 (+/-) transgenic mouse assay in this case. The p53 (+/-) transgenic mouse assay is primarily useful in evaluation of genotoxicity positive compounds, however, there was an absence of positive genotoxicity findings in iron-supplemented animals, the proposed test design. In addition, the sponsor's dose selection did not appear to be based on any demonstrable end point (doses in excess of those tested in the dose ranging studies were proposed and the body weight effects observed for females did not appear dose-related). The committee had no basis to predict the toxicity of the proposed high dose (600 mg/kg), as this dose has not been tested in iron-supplemented mice. Therefore, the committee was uncertain about the survival of the animal at this dose level for the span of the carcinogenicity study.

Executive CAC Recommendations and Conclusions:

- I. The committee did not concur with the sponsor's proposed doses of 30, 100, 300, and 600 mg/kg/day.

2. The appropriateness of the 26-week p53 (+/-) transgenic mouse assay was discussed and was recommended as appropriate for discussion at the PTCC meeting given the absence of positive genotoxicity findings in the iron-supplemented animals.
3. The possibility of the inclusion of non-iron-supplemented animals in the proposed carcinogenicity study was also discussed.

Note: The protocol and the dose selection were discussed by the Pharmacology Toxicology Coordinating Committee (PTCC) on August 16, 2001. The majority considered that the positive genotoxicity results of the micronucleus assays in non-iron supplemented rats are relevant. Therefore, the committee recommended that it is appropriate to conduct p53 (+/-) transgenic mouse assay in both iron supplemented and non-iron supplemented animals, which would help in the interpretation of overall carcinogenic potential of the drug. Inclusion of at least one treatment group without supplementation was recommended.

The committee could not recommend the doses for the non-iron supplemented animals, as there was no data available in non-iron supplemented C57BL/6 mice. However, the committee felt that the doses would likely be lower for the non-iron supplemented mice because of the observed greater toxicity of ICL 670 in other rodents on non-iron supplemented diet.

The PTCC did believe that the doses tested in the male mice in the dose ranging study that yielded decreases in bodyweight gain (200 mg/kg) could be considered an MTD if reproducible, but could not recommend doses for iron-supplemented female mice.

It was felt that kinetics could be collected at study termination using the same animals for tumor analysis, and that the additional satellite treated animals were unnecessary.



Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD-180
/TChakraborti, HFD-180
/JChoudary, HFD-180
/CSO/PM, HFD-181
/AScifried, HFD-024

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Joseph DeGeorge
8/21/01 11:34:34 AM

APPENDIX-4

ExecCAC Meeting Minutes Dated September 19, 2002 for Mouse Carcinogenicity Study

Appears This Way
On Original

Executive CAC

September 17, 2002

Committee: David Morse, Ph.D., HFD-150, Acting Chair
Alex Jordan, Ph.D., HFD-580, Alternate Member
Frank Sistare, Ph.D., HFD-910, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the P53 study, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias
This drug is currently under Phase II clinical trial.

P 53 Mouse Carcinogenicity Study Protocol and Dose Selection:

The sponsor has submitted a protocol and dose selection for a 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 20, 100 and 200 mg/kg/day for iron-supplemented animals and 20 mg/kg/day for non-iron-supplemented animals. The sponsor's dose selection was based on the results of the 4-week dose ranging study (study no. 0170121) in C57BL/6 mice with and without iron-supplementation. The sponsor did not submit the actual data or full report of this study. Only summary statements were made.

In the 4-week dose ranging study (study no. 0170121), iron-supplemented C57BL/6 (wild-type) mice were treated with ICL-670 by oral gavage at 100, 300 and 600→450 mg/kg/day. In case of iron-supplemented animals, due to excessive mortality/moribundity

at 600 mg/kg/day following two doses, dosing was suspended on Days 3 – 7 and resumed on Day 8 at 450 mg/kg/day. The animals with standard diet (non-iron-supplemented) were treated at 100 and 600 mg/kg/day.

Iron-Supplemented Diet:

There was no mortality at 100 or 300 mg/kg/day in either sex. However, test article-related mortality was observed in both sexes (three males and one female) at 450 mg/kg/day. The causes of deaths were not discussed. Treatment-related histopathological changes were predominantly observed in males in the following organs at 600→450 mg/kg/day: stomach (gastric erosions and/or ulcers and inflammation in both sexes), cecum (necrosis of epithelial cells in crypts of the cecum), jejunum (hemorrhage), liver (necrosis; however, there was no discussion about the type of necrosis i.e., single cell or focal), adrenal cortex (cytoplasmic vacuolation), lymphoid organs (lymphoid depletion in both sexes, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes), spleen (extramedullary hematopoiesis) and kidney (vacuolation, necrosis and regenerative changes in both sexes and proteinaceous casts). Hematology (increase in hemoglobin in both sexes, hematocrit in males and reticulocyte in males) and serum chemistry (calcium, phosphorus, cholesterol and triglyceride concentrations were increased in males) changes were observed at 300 mg/kg/day. However, the magnitudes of these changes could not be calculated in the absence of any data. In addition, extensive iron-depletion was observed in the liver (83% and 43% reduction in males and females, respectively) and kidney (34% and 46% reduction in males and females, respectively). Based on the mortality and histopathological changes at 600→450 mg/kg/day, the maximum tolerated dose (MTD) in iron-supplemented male mice was considered as 200 mg/kg/day. This MTD estimation is also in agreement with the previous Exec CAC recommendations (August 7, 2001). However, the MTD in females could not be determined in the absence of complete data and full report of this study.

Standard Diet:

There was no mortality in females at 100 mg/kg/day; however, mortality was observed in males at 100 mg/kg/day and at 600 mg/kg/day, all of the animals died or were sacrificed in moribund on Days 3 or 4, following 2 daily doses of ICL-670. The causes of deaths were also not mentioned. Treatment-related histopathological changes were observed in the following organs in both sexes at both doses: stomach (gastric erosions and/or ulcers and inflammation), cecum (necrosis of epithelial cells in crypts of the cecum), jejunum (hemorrhage), liver (necrosis, periportal inflammation containing fibrous tissue and bile duct hyperplasia), lymphoid organs (lymphoid depletion in all animals, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes), spleen (extramedullary hematopoiesis), heart (multifocal myocytolysis) and kidney (vacuolation, necrosis and regenerative changes and proteinaceous casts). The MTD in males appeared to be < 100 mg/kg/day based on mortality and histopathological changes at this dose. The MTD in females appeared to be < 600 mg/kg/day based on mortality at this dose. However, an accurate estimation of MTD in either sex could not be made due to the absence of lower dose levels below 100 mg/kg/day in males and 600 mg/kg/day in females.

Executive CAC Recommendations and Conclusions:

1. The committee concurred with the sponsor's proposed high dose of 200 mg/kg/day for iron-supplemented males. This concurrence was based on the estimation of an MTD from the previous full study (study no. 981060) information and on the results of the current dose ranging study (study no. 0170121) showing mortality at 450 mg/kg/day, which was slightly more than twice the proposed high dose.
2. The Committee did not concur with proposed doses for iron-supplemented females in the absence of a full report of the dose ranging study and complete data. The sponsor should provide full report of the dose ranging study (study no. 0170121) with complete line listing of data.
3. The committee did not concur with the proposed doses for non-iron-supplemented mice due to an inadequate range of doses having been tested in the range finding study. It was recommended that the sponsor should conduct an additional dose ranging study in non-iron-supplemented mice at doses below 100 mg/kg/day in males and between 100 and 600 mg/kg/day in females.

The sponsor should consider conducting histopathological examination in all dose groups. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the control and high dose animals, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group. We note that given the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately or completely described in the recommendations by McConnell et al.

David Morse, Ph.D.
Acting Chair, Executive CAC

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/JChoudary, HFD-180
/TChakraborti, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

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/s/

David Morse
9/19/02 05:03:29 PM

APPENDIX-5

ExecCAC Meeting Minutes Dated November 14, 2005 for the Mouse Carcinogenicity Study

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Executive CAC

November 12, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Josie Yang, Ph.D., HFD-550, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the P53 study, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias
This drug is currently under Phase II clinical trial.

P 53 Mouse Carcinogenicity Study Protocol and Dose Selection:

The sponsor has presented a revised protocol and dose selection for the 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 20, 100 and 200 mg/kg/day for iron-supplemented males and 30, 100 and 300 mg/kg/day for iron-supplemented females. In this protocol, the sponsor proposed to use 30 and 100 mg/kg/day for males and females on standard diet, respectively. In case of C57BL/6 mice (wild-type), the sponsor proposed to use 200 and 300 mg/kg/day in iron-supplemented males and females, respectively. Sponsor's dose selection was based on the MTD established in the oral 4-week dose ranging study (study no. 0170121) in iron-supplemented C57BL/6 mice at 100, 300 and 600→450 mg/kg/day and in C57BL/6 mice on standard diet at 100 and 600 mg/kg/day. It is to be mentioned here that the sponsor presented the full report of this study in this submission.

In the 4-week dose ranging study (study no. 0170121), iron-supplemented C57BL/6 (wild-type) mice were treated with ICL-670 by oral gavage at 100, 300 and 600→450 mg/kg/day. In case of iron-supplemented animals, due to excessive mortality/moribundity at 600 mg/kg/day following two doses, dosing was suspended on Days 3 – 7 and resumed on Day 8 at 450 mg/kg/day. The animals with standard diet (non-iron-supplemented) were treated at 100 and 600 mg/kg/day.

Iron-Supplemented Diet:

There was no mortality at 100 or 300 mg/kg/day in either sex. However, test article-related mortality was observed in both sexes (three males and one female) at 450 mg/kg/day. The causes of deaths were not discussed. In males, the body weight gain was suppressed by 8%, 18% and 84% at 100, 300 and 600→450 mg/kg/day, respectively, compared to control weight gain. In contrast, the body weight gain in females was increased by 9%, 23% and 64% at 100, 300 and 600→450 mg/kg/day, respectively, when compared to control weight gain. Treatment-related histopathological changes were predominantly observed in males in the following organs at 600→450 mg/kg/day: stomach (gastric erosions and/or ulcers and inflammation in both sexes), cecum (necrosis of epithelial cells in crypts of the cecum in 1 of 10 male), jejunum (hemorrhage in one male), liver (necrosis in 3 of 10 males; necrosis either occurred focally or was seen in scattered hepatocytes throughout liver lobules), adrenal cortex (cytoplasmic vacuolation in 2 of 10 males), lymphoid organs (lymphoid depletion in 6 of 10 males, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 2 of 10 males), spleen (extramedullary hematopoiesis in 2 of 10 animals of each sex), and kidney (tubular necrosis and regenerative changes in the cortex of 8 of 10 males and 1 of 10 females and proteinaceous casts in one male). The MTD in iron-supplemented males appeared to be 200 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day and severe iron depletion (85%) at ≥ 300 mg/kg/day. However, in case of females, the MTD may be considered as 300 mg/kg/day based on the mortality and histopathological changes (gastric erosions and/or ulcers and inflammation in the glandular stomach, extramedullary hematopoiesis in the spleen, and necrosis and regenerative changes in the kidney) at 600→450 mg/kg/day. Although iron depletion was also observed in the liver and kidney at 300 mg/kg/day in females, however, the extent of this iron depletion is much more severe in males when compared to females (43% iron depletion in the liver in females compared to 83% in males at 300 mg/kg/day).

Standard Diet:

There was no mortality in females at 100 mg/kg/day; however, six males died between Days 13 and 25 and one male was sacrificed in moribund condition on Day 14. At 600 mg/kg/day, all of the animals died or were sacrificed in moribund on Days 3 or 4, following 2 daily doses of ICL-670. The causes of deaths were not mentioned. The body weight gain was suppressed by 92% in males at 100 mg/kg/day compared to control. In contrast, in females, body weight gain at 100 mg/kg/day was similar to control weight gain. In males, serum alanine amino transferase (494% of control, control = 35 U/l), aspartate amino transferase (AST, 184% of control, control = 126 U/l), and alkaline phosphatase (ALP, 382% of control, control = 86 U/l) values were increased at 100

mg/kg/day compared to control. Histopathological changes were observed in the stomach (gastric erosions and/or ulcers and inflammation in males at 100 mg/kg/day and in both sexes at 600 mg/kg/day), cecum (necrosis of epithelial cells in crypts of the cecum in 2 of 10 males and 3 of 10 females at 600 mg/kg/day), liver (necrosis in 8 of 10 males and 2 of 10 females at 100 mg/kg/day, periportal inflammation containing fibrous tissue and bile duct hyperplasia in most males and in one female at 100 mg/kg/day), lymphoid organs (lymphoid depletion in all males at $\geq$ 100 mg/kg/day and 7 of 10 females at 600 mg/kg/day, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 5 of 10 and 6 of 10 males, respectively, and in 2 of 10 and 3 of 10 females at 600 mg/kg/day, respectively), spleen (extramedullary hematopoiesis in 2 of 9 males at 100 mg/kg/day), heart (multifocal myocytolysis in the ventricle in 4 males and 1 female at 600 mg/kg/day) and kidney (vacuolation, necrosis and regenerative changes in 9 of 10 males at 100 mg/kg/day and in 1 of 10 females at 600 mg/kg/day and proteinaceous casts in two males at 100 mg/kg/day). It is to be mentioned here that the histopathological changes at 100 mg/kg/day were predominantly observed in males. The MTD in males appeared to be <100 mg/kg/day based on the mortality and histopathological changes at 100 mg/kg/day. However, in case of females, the MTD may be considered as 100 mg/kg/day based on the mortality at 600 mg/kg/day and absence of any significant histopathological changes at 100 mg/kg/day in females.

Executive CAC Recommendations and Conclusions:

p53 (+/-) Transgenic Mice

1. Iron-Supplemented Diet:

Males: The committee previously concurred with the sponsor's proposed doses of 20, 100 and 200 mg/kg/day for males.

Females:

Based on the MTD derived from observed mortality and histopathological lesions at 450 mg/kg/day, the committee concurred with the sponsor's proposed doses of 30, 100 and 300 mg/kg/day for females.

2. Standard Diet:

Males:

The committee concurred with the proposed dose of 30 mg/kg/day for males based on the observed mortality and histopathology findings at 100 mg/kg/day.

Females:

Based on the MTD derived from observed mortality and histopathological lesions at 600 mg/kg/day, the committee concurred with the sponsor's proposed dose of 100 mg/kg/day for females.

3. C57BL/6 Mice:

Males: The committee concurred with the proposed dose of 200 mg/kg/day for iron-supplemented males.

Females: The committee concurred with the proposed dose of 300 mg/kg/day for iron-supplemented females.

The sponsor should consider conducting histopathological examination in all dose groups. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the control and high dose animals, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group. We note that given the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately or completely described in the recommendations by McConnell et al.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/CSO/PM, HFD-181
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/s/

Joe Contrera
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APPENDIX-6

Neo-Plastic and Non-Neoplastic Findings in the p53 Heterozygous Mouse Carcinogenicity Study

GROUP 1 to 7 (MALES)

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Table 3.2
 ICL678: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:11
 Page : 1
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron			Non-supplemented Feed		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
CARDIOVASCULAR SYSTEM							
HEART		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	20	21	18	20	23
Atrophy, myocardium,							
minimal							1
Score Expanded Totals							1
Degeneration, myocardium,							
focal,							
minimal					1		
Score Expanded Totals					1		
Fibrosis, subendocardial,							
focal,							
slight		1					
Score Expanded Totals		1					
Inflammation, epicardium,							
focal,							
slight		1			1		
Score Expanded Totals		1			1		
Inflammation, myocardium,							
focal,							
minimal		1				1	
Score Expanded Totals		1				1	
LYMPHOMA [N].			1				
Mineralization, myocardium,							
focal,							
minimal			1				1
Score Expanded Totals			1				1
Vasculatation, myocardium,							
minimal		1					
Score Expanded Totals		1					

[N] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-NAR-04 13:11
 Page : 2
 PLACBS 2800 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
CARDIOVASCULAR SYSTEM							
HEART		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, myocardium, focal.							
minimal		5	3	4	4	4	3
slight					2		
Score Expanded Totals		5	3	4	6	4	3
AORTA		(25)	(25)	(25)	(24)	(25)	(25)
Within normal limits		25	25	25	24	25	25
ARTERY		(1)					
Polyarteritis.							
slight		1					
Score Expanded Totals		1					
DIGESTIVE SYSTEM							
SALVIVARY GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		13	4	11	11	10	13
Granules, serous, decreased.							
moderate			1				
Score Expanded Totals			1				
Infiltrate, lymphocyte.							
minimal		13	20	13	14	13	10
slight		1		1		2	2
Score Expanded Totals		12	20	14	14	15	12
Mineralization, duct.							
minimal			1				
Score Expanded Totals			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:11
 Page : 3
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Food with Iron			Non-supplemented Food		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM							
SALIVARY GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Necin, sublingual, increased, diffuse, slight							
Score Expanded Totals							1 1
TONGUE		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	24	25	25	25	24
Inflammation, focal, chronic, minimal			1				
Score Expanded Totals			1				
Inflammation, artery, moderate		1					
Score Expanded Totals		1					
ESOPHAGUS		(25)	(25)	(25)	(25)	(25)	(24)
Within normal limits		25	25	24	24	25	25
Degeneration, muscularis, moderate				1			
Score Expanded Totals				1			
Degeneration, muscularis, slight					1		
moderate					1		
Score Expanded Totals				1	1		
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		7	11	11	9	11	8
Crystal, limiting ridge, minimal			3				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-NAR-04 13:11
 Page : 4
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron			Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Crystal, limiting ridge. Score Expanded Totals			3				1
Cyst, glandular stomach. minimal Score Expanded Totals		1				1	
Cyst, limiting ridge. minimal slight Score Expanded Totals		1	5	3	1	5	5
Dilatation, gland, focal. minimal slight Score Expanded Totals		1	5	3	2	5	5
Edema, nonglandular. slight Score Expanded Totals		1		1	1	1	2
Erosion, glandular. minimal slight Score Expanded Totals		6	4	4	4	4	6
Hyperkeratosis. minimal Score Expanded Totals		1	4	1	4	1	2
Infiltrate, lymphocyte. minimal Score Expanded Totals		7	4	5	8	5	9
Inflammation, glandular, mixed cell, focal. minimal							1
		14	4	6	9	7	7
							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

5-MAR-04 13:11
 Page : 5
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented Feed			
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day	
DIGESTIVE SYSTEM								
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, glandular, mixed cell, focal, slight				3	1			1
Score Expanded Totals		14	4	9	10	7	7	2
Inflammation, submucosa, non-glandular, chronic, minimal		2	3	2	2			1
Score Expanded Totals		2	3	2	3			1
LYMPHOMA [M], Metaplasia, limiting ridge, minimal		2	4	1	1	2	1	
Score Expanded Totals		2	4	1	1	2	1	
Mineralization, glandular, mucosa, focal, minimal			1					
Score Expanded Totals			1					
Vacuolation, epithelium, glandular, minimal								1
Score Expanded Totals							1	1
Vacuolation, epithelium, glandular, focal, minimal					2			
Score Expanded Totals					2			

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117

5-MAR-04 13:11
 Page : 6
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Isom				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM								
DUODENUM		(25)	(25)	(25)	(25)	(25)	(25)	(24)
Within normal limits		25	24	24	25	24	25	24
Dilatation, Brunner's gland.								
minimal						1		
slight				1				
Score Expanded Totals				1		1		
LYMPHOMA [M]			1					
JEJUNUM		(25)	(25)	(25)	(25)	(25)	(25)	(24)
Within normal limits		24	24	22	23	24	25	23
Hyperplasia, lymphoid tissue.								
minimal		1	1	1	1	1		1
slight				2	1			
Score Expanded Totals		1	1	3	2	1		1
ILEUM		(25)	(25)	(25)	(25)	(25)	(25)	(23)
Within normal limits		24	24	23	22	23	18	22
Congestion, mucosa.								
minimal							1	
Score Expanded Totals							1	
Hyperplasia, lymphoid tissue.								
minimal		1		1	2		4	1
slight				1	1	1	1	
Score Expanded Totals		1		2	3	1	5	1
Inflammation, mixed cell.								
slight						1		

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0170117
 5-NAR-04 13:11
 Page : 7
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	0-oxazidine 400 ng/kg/day
DIGESTIVE SYSTEM							
ILEUM		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell.							
Score Expanded Totals						1	
LYMPHOMA [N]			1				
Necrosis, lymphoid tissue.							
minimal						1	
Score Expanded Totals						1	
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		17	18	17	15	25	24
Hyalinization, lymphoid tissue.							
minimal			1		1		
Score Expanded Totals			1		1		
Hyperplasia, crypt.							
minimal		4	3	3	7		
Score Expanded Totals		4	3	3	7		
Hyperplasia, lymphoid tissue.							
minimal		2		1	1		1
slight			1				
Score Expanded Totals		2	1	1	1	1	1
Inflammation, mixed cell.							
minimal		2	1	2	1		
Score Expanded Totals		2	1	2	1		
LYMPHOMA [N]			1				
Necrosis, single cell, epithelium.							
minimal		5	5	6	7		

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q270117

5-MAR-04 13:11
 Page : 8
 PLACES 2080 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM							
CECUM		(25)	(25)	(25)	(25)	(25)	(25)
Necrosis, single cell, epithelium.							
Score Expanded Totals		5	5	6	7		
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	24	25	25	25	24
Hyperplasia, crypt. minimal			1				
Score Expanded Totals			1				
Hyperplasia, lymphoid tissue. minimal		1					1
Score Expanded Totals		1					1
Inflammation, mixed cell. slight			1				
Score Expanded Totals			1				
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		23	24	24	23	24	24
Congestion, mucosa, focal. minimal						1	
Score Expanded Totals						1	
Dilatation, crypt, lymphoid tissue. minimal							1
Score Expanded Totals							1
Hyperplasia, lymphoid tissue. minimal		2	1	1	2	1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron			Non-supplemented Feed		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM							
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
Score Expanded Totals		2	1	1	2	1	1
MESENTERY						(1)	
Inflammation, mixed cell.							
minimal						1	
Score Expanded Totals						1	
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		5	10	7	7	5	4
Atrophy, exocrine, focal.							
minimal		1				1	1
Score Expanded Totals		1				1	1
Eosinophilia, acinar cell.							
focal.							
minimal		3	1	1		2	
Score Expanded Totals		3	1	1		2	
Dilatation, duct.							
minimal						1	
Score Expanded Totals						1	
Hyaline bodies, acinar cell, focal.							
minimal		2			1		
Score Expanded Totals		2			1		
Hyperplasia, islet cell.							
minimal		13	14	17	15	13	4
slight		1					
Score Expanded Totals		14	14	17	15	13	4

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Irrm				Non-supplemented Feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-creosidine 400 ng/kg/day	
DIGESTIVE SYSTEM									
PANCREAS									
Hypertrophy, acinar, focal.		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
minimal		2			1	1		2	
slight				1	1				
Score Expanded Totals		2		1	2	1		2	
Infiltrate, lymphocyte.		6	5	2	3	3	4	9	
minimal		6	5	2	3	3	4	9	
Score Expanded Totals		6	5	2	3	3	4	9	
Inflammation, chronic active, focal.				1					
slight				1					
Score Expanded Totals				1					
LYMPHOMA (M)									
Necrosis, exocrine, focal.			1						
minimal		1							
slight					1				
Score Expanded Totals		1			1				
Synogen decreased.									
minimal		4	2	5	3	7	7	11	
slight								2	
Score Expanded Totals		4	2	5	3	7	7	13	
Decrease, islets of Langerhans.									
minimal		2				1	3	1	
slight		1							
Score Expanded Totals		3				1	3	1	

[N] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented Feed			
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-erasidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM								
GALL BLADDER		(25)	(25)	(24)	(25)	(25)	(24)	(25)
Within normal limits		20	23	19	22	23	22	23
Hyaline bodies, cytoplasm, epithelium.								
slight							1	
Score Expanded Totals							1	
Infiltrate, lymphocyte.								
minimal		1		1				
slight								1
Score Expanded Totals		1		1				1
Inflammation, lamina propria, mixed cell.								
minimal		3	1	2	1	2	1	
slight		1		2				1
Score Expanded Totals		4	1	4	3	2	1	1
LYMPHOMA [M].			1					
LIVER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits			1	2		1	1	
Angiectasis, focal.								
minimal		1					1	
Score Expanded Totals		1					1	
Bile stasis.								
minimal								15
Score Expanded Totals								15
Focus, basophilic.								
marked					1			
Score Expanded Totals					1			

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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		INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
ORGAN/TISSUE Finding	TREATMENT	Feed with Irrm				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM								
LIVER								
Glycogen, decreased, hepatocyte.		(25)	(25)	(25)	(25)	(25)	(25)	(25)
slight							1	
Score Expanded Totals							1	
Hyperplasia, bile duct.								1
minimal								1
Score Expanded Totals								1
Hyperplasia, regenerative.								1
slight								1
Score Expanded Totals								1
Hypertrophy, centrilobular.								
minimal		14	12	16	19	7	10	
slight		3	1	4	3			
moderate								
Score Expanded Totals		17	13	20	22	7	10	6
Hypertrophy, midzonal.								6
slight								1
moderate								16
marked								2
Score Expanded Totals								19
Inclusion, intranuclear, cytoplasmic inclusion.								
minimal		1	1					
Score Expanded Totals		1	1					
Inclusion, intranuclear, cytoplasmic vacuolation.								
slight								1
Score Expanded Totals								1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented Feed			
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	20 ng/kg/day	p-cresidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inclusion, intranuclear, invaginated cytoplasm.								
minimal								1
slight								1
Score Expanded Totals								2
Inclusion, intranuclear, cytoplasmic invagination.								
minimal		4	1	1	2	2	1	6
slight								14
moderate								1
Score Expanded Totals		4	1	1	2	2	1	21
Inclusion, intranuclear, hyaline.								
minimal				1			1	
Score Expanded Totals				1			1	
Infarct.								
slight				1				1
Score Expanded Totals				1				1
Inflammation, lymphocyte, perivascular.								
minimal		14	7	8	10	15	7	1
slight			1	1	1	1	2	
Score Expanded Totals		14	8	9	11	16	9	1
Inflammation, mixed cell, focal.								
minimal		4	4	5	1	8	10	5
slight							1	
Score Expanded Totals		4	4	5	1	8	11	5

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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		INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-eresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
LYMPHOMA [M].			2					
Mitotic figures, abnormal.								
minimal								1
Score Expanded Totals								1
Mitotic figures, increased.								
minimal								3
Score Expanded Totals								3
Necrosis, centrilobular.								
slight								1
Score Expanded Totals								1
Necrosis, hepatocellular.								
focal.								
minimal		1	4	2		5	7	1
Score Expanded Totals		1	4	2		5	7	1
Necrosis, hepatocyte.								
single cell.								
minimal								6
slight								16
moderate								1
Score Expanded Totals								23
Necrosis, midzonal.								
minimal								8
slight								1
Score Expanded Totals								9
Pigment, hepatocellular.								
minimal								12
Score Expanded Totals								12

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IC167F: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	β-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Pigment, Kupffer cell.							
minimal							5
slight							20
Score Expanded Totals							25
Tension focus.							
minimal			2	2	3	4	2
slight	1		1	2	1	1	1
Score Expanded Totals	1		3	2	4	5	2
TRANSITIONAL CELL CARCINOMA (M), metastatic site.							1
Vacuolation, hepatocellular, centrilobular.							
minimal	1	1	3	6	9	6	
slight	1	3	11	5	1		
moderate	2	2	1	4	1		
Score Expanded Totals	6	6	15	15	11	6	
Vacuolation, hepatocellular, midzonal.							
minimal		1					
Score Expanded Totals		1					
Vacuolation, hepatocellular, panlobular.							
minimal	5	8	3		2	2	
slight	5	6	3				
Score Expanded Totals	14	12	6		2	2	

(N) Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICI670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Ixam			Non-supplemented Feed		
	0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-arsidine 400 mg/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, hepatocellular, periportal.							
minimal		1	1	1	1		
Score Expanded Totals		1	1	1	1		
Extrasinusoidal hematopoiesis.							
minimal		12	11	15	12	11	13
Score Expanded Totals		12	11	15	12	11	13
Multinucleated giant cell.							
minimal							1
Score Expanded Totals							1
ENDOCRINE SYSTEM							
ADRENAL		(24)	(25)	(25)	(25)	(25)	(25)
Within normal limits		9	13	11	13	19	16
Accumulation, foamy, macrophage.							
moderate							1
Score Expanded Totals							1
Atrophy, cortex.							
minimal		4	3	3	3	1	1
slight			1			1	
Score Expanded Totals		4	4	3	3	2	1
Dilatation, vein.							
minimal		4		4	4		
slight			1				
Score Expanded Totals		4	1	4	4		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICHS70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Food with Iron			Non-supplemented Feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-irresidine 400 ng/kg/day
ENDOCRINE SYSTEM								
ADRENAL		(24)	(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, cortex, focal.								
moderate		1						
marked			1	2				
Score Expanded Totals		1	1	2				
Hyperplasia, medulla, focal.								
minimal							1	
marked						1		
Score Expanded Totals						1	1	
Hyperplasia, subcapsular cell.								
minimal		3	1	3	3	3	2	2
Score Expanded Totals		3	1	3	3	3	2	2
Hypertrophy, cortex, focal.								
minimal		3	2	4	3		1	1
slight		1	3	2	1	1	2	
moderate		2		1		1	1	1
marked		2	3	2	1			1
Score Expanded Totals		8	8	9	5	2	4	3
LYMPHOMA [M].								
PHENCHRONOCYTOMA [B].			1					1
Extracapsular cortical tissue.								
moderate								1
Score Expanded Totals								1
CORTICAL ADENOMA [B].				1		1	1	1

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 TCE670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Food with Irrm			Non-supplemented Food		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
ENDOCRINE SYSTEM							
PARATHYROID							
Within normal limits	(22)						
Dilatation, vein.	20		(21)	21	(20)	22	(19)
slight	1						15
Score Expanded Totals	1						22
Hypertrophy, focal.							
minimal	1			1			
slight	1			1			
Score Expanded Totals	1			1			
PITUITARY							
Within normal limits	(25)		(25)	(25)	(25)	(23)	(24)
Cyst, pars distalis.	19		19	21	23	22	22
minimal	6	2	4	2	3	1	1
slight	1	1	4	2	3	1	1
Score Expanded Totals	6	3	4	2	3	1	2
Infiltrate, lymphocyte, pars distalis, focal.							
minimal			1				
slight		1	1				
Score Expanded Totals		1	1				
LYMPHOMA (N)							
			2				
THYROID							
Within normal limits	(25)	(25)	(24)	(25)	(25)	(25)	(24)
Cyst, follicular.	23	25	22	24	24	24	21
minimal			1				
Score Expanded Totals			1				

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICLE78: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCES OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Iron			Non-supplemented Feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-arsidine 400 ng/kg/day	
ENDOCRINE SYSTEM									
THYROID									
Ectopic thymus.		(25)	(25)	(24)	(25)	(25)	(25)	(24)	
minimal		1							
Score Expanded Totals		1							
Hyperplasia, c-cell, focal.				1	1				
minimal				1	1				
Score Expanded Totals				1	1				
Hyperplasia, follicular, focal.									1
minimal									1
Score Expanded Totals									1
Infiltrate, lymphocyte.						1	1		3
minimal						1	1		3
Score Expanded Totals						1	1		3
Inflammation, artery.		1							
moderate		1							
Score Expanded Totals		1							
Neurosis, fibrinoid, artery.		1							
slight		1							
Score Expanded Totals		1							
GENITOURINARY SYSTEM									
SPIDIDYMIDES									
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Degeneration, epithelium, focal.		18	20	17	22	16	19	21	
minimal					1				
slight		1							

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM							
EPIDIDYMIDES		(25)	(25)	(25)	(25)	(25)	(25)
Degeneration, epithelium, focal.							
Score Expanded Totals		1			1		
Dilatation, lumen.						1	
Score Expanded Totals						1	
Fibrosis, interstitium.					1		
minimal							1
Score Expanded Totals					1		1
Infiltrate, lymphocyte.		5	3	3	1	5	3
minimal						1	
Score Expanded Totals		5	3	5	1	6	3
Inflammation, interstitium.					1		
minimal		1					
Score Expanded Totals		1			1		1
Lymphangiectasia.							
minimal		1		3			
Score Expanded Totals		1		1		1	
LYMPHOMA (H).			1	4			
Oligospermia.							
moderate		1			1	2	
marked						1	
Score Expanded Totals		1			1	3	2

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Ixxm			Non-supplemented Feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
EPIDIDYMIDES								
Vacuolation, epithelium, increased.		(25)	(25)	(25)	(25)	(25)	(25)	(25)
minimal		1	1	2	2	2	3	
slight		1						
Score Expanded Totals		2	1	2	2	2	3	
LEIOMYOSARCOMA [M], PNL.								
KIDNEY								
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Atrophy, tubular, focal.			1	7	2	1	4	
minimal		2						4
slight								5
moderate								11
marked								21
Score Expanded Totals		2						
Atrophy, tubular, diffuse.								1
moderate								1
Score Expanded Totals								
Basophilic tubules.								
minimal		10	8	8	15	7	14	6
slight							1	7
moderate								1
marked								14
Score Expanded Totals		10	8	8	15	7	15	
Cast, proteinaceous.								
minimal		4	2	2	8	12	4	11
slight								8
moderate								

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented Feed			
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-cresidine 400 mg/kg/day
GENITOURINARY SYSTEM								
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Cast, proteinaceous.								
Score Expanded Totals		4	9	2	8	12	4	19
Deposition, fat, glomerulus.								
minimal		1	2		1			2
Score Expanded Totals		1	2		1			2
Dilatation, tubular.								
minimal							1	3
slight								1
Score Expanded Totals							1	4
Dilatation, vein, pelvis.								
minimal					1			
slight					1		1	
Score Expanded Totals					2		1	
Ectopic tissue, bone, glomerulus.								
minimal							1	
Score Expanded Totals							1	
Edema, pelvis.								
slight								1
Score Expanded Totals								1
Fibrosis, interstitial, focal.								
minimal		1						3
slight								4
Score Expanded Totals		1						7
Fibrosis, interstitial, diffuse.								
minimal								1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Fibrosis, interstitial, diffuse.							1
Score Expanded Totals							1
Hyalinization, glomeruli.							
minimal		1	1	2	3	2	1
slight							2
Score Expanded Totals		1	3	2	3	2	1
Hydronephrosis.							
minimal		1			2		
slight							2
moderate							2
marked							1
Score Expanded Totals		1			2		5
Hyperplasia, epithelial, pelvis.							
slight							1
Score Expanded Totals							1
Infiltrate, lymphocyte.							
minimal		10	6	8	9	11	9
slight			2	6	4	2	2
moderate							8
marked							1
Score Expanded Totals		10	8	14	13	13	21
Inflammation, mixed cell, focal.							
minimal							1
Score Expanded Totals							1
Karyomegaly, papilla.							
Slight							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-oxazolidinone 400 mg/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Hypertrophy, papilla.							
Score Expanded Totals							1
LYMPHOMA [N]			2				
Mineralization, focal.							
minimal		4	4	3	4	8	8
slight							9
moderate							15
marked							1
Score Expanded Totals		4	4	3	4	8	25
Necrosis, epithelium, tubular, focal.							
minimal							1
Score Expanded Totals							1
Necrosis, papilla.							
slight							4
moderate							11
Score Expanded Totals							15
Vacuolation, tubular, epithelium, focal.							
minimal		18	15	3		18	1
slight		5	1			3	1
Score Expanded Totals		23	16	3		21	2
Vacuolation, tubular, epithelium, diffuse.							
minimal							1
Score Expanded Totals							1
Increase, mesangial cell.							
minimal					2		

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Iron			Non-supplemented Feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-oxidized 400 ng/kg/day	
GENITOURINARY SYSTEM									
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Increase, mesangial cell.									
Score Expanded Totals					2				
PROSTATE		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		10	11	11	8	13	9	14	
Cleft, cholesterol, focal, minimal		1			1	1			
Score Expanded Totals		1			1	1			
Distasia									
minimal				1					
slight							1		
Score Expanded Totals				1			1		
Fibrosis, interstitial, focal, minimal				1				2	
slight							1		
Score Expanded Totals				1			2		
Fibrosis, lumen, focal, minimal					1	1	1		1
slight		1							
Score Expanded Totals		1			1	1	1		1
Hyperplasia, epithelium, focal, minimal			1		1	1	2		
slight			1		1	1	2		
Score Expanded Totals			2		2	2	4		
Infiltrate, lymphocyte, minimal		8	7	11	7	5	11		3
slight			3		1	3	2		3
moderate					1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-arsidine 400 ng/kg/day
GENITOURINARY SYSTEM								
PROSTATE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Infiltrate, lymphocytic.								
Score Expanded Totals		8	10	11	9	8	13	6
Infiltrate, lymphocytic, ganglionic.								
minimal			1					
slight		1	1	1				1
moderate			1					
Score Expanded Totals		1	3	1				1
Inflammation, mixed cell.								
minimal					1		1	
slight							1	1
moderate							1	
Score Expanded Totals					1		2	1
TRANSITIONAL CELL CARCINOMA								
(M), metastatic site.								2
Content, inspissated, focal.								
minimal		3	3	3	4	4	6	3
slight		4	2	1	4	1	4	
Score Expanded Totals		7	5	4	8	5	10	3
SEMINAL VESICLE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		9	15	14	9	17	11	14
Dilatation.								
slight		1						
Score Expanded Totals		1						

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027011

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 PLACES 2100 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS							
		Males							
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed			
		0	20	100	200	0	30	β-cresidine	400
		ng/kg/day	ng/kg/day	ng/kg/day	ng/kg/day	ng/kg/day	ng/kg/day	ng/kg/day	ng/kg/day
GENITOURINARY SYSTEM									
SEMINAL VESICLE									
Dilatation, coagulating gland.									
		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
	minimal	15	8	8	12	8	6	7	
	slight		2	3	4		6	4	
	Score Expanded Totals	15	10	11	16	8	12	11	
Hypertrophy, smooth muscle.									
	slight	1							
	Score Expanded Totals	1							
Infiltrate, lymphocyte.									
	minimal						2		
	slight							1	
	Score Expanded Totals						2	1	
Inflammation, mixed cell.									
	moderate						1		
	Score Expanded Totals						1		
Secretion reduced.									
	slight						1		
	Score Expanded Totals						1		
TESTIS									
	Within normal limits	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
	Regeneration, seminiferous tubule.	23	21	24	23	19	24	20	
	slight					1		1	
	moderate	1			1	2		1	
	Score Expanded Totals	1			1	3		2	
Dilatation, seminiferous tubule.									
	minimal		2			2	1	2	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

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 VLABS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 100 ng/kg/day
GENITOURINARY SYSTEM								
TESTIS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Dilatation, seminiferous tubule.								
slight								1
moderate		1			1	3		1
Score Expanded Totals		1	2		1	5	1	4
Granuloma, spermatic.								
moderate		1						
marked						1		
Score Expanded Totals		1				1		
Hyperplasia, interstitial cell, focal.								
minimal			2		1	1		
slight		1						
Score Expanded Totals		1	2		1	1		
Infiltrate, lymphocyte.								
minimal				1				
Score Expanded Totals				1				
Mineralization, focal.								
minimal		1						1
slight						3		
Score Expanded Totals		1				3		1
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		20	22	24	23	22	21	
Acanthosis, epithelium.								
minimal								7
slight								9
moderate								4
marked								1

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM							
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)
Acanthosis, epithelium, Score Expanded Totals							21
Atypia, epithelium, focal, minimal							5
slight							10
moderate							1
Score Expanded Totals							16
Hyperplasia, transitional cell, diffuse, minimal		2	1	1	2	3	2
slight							10
moderate							14
Score Expanded Totals		2	1	1	2	3	25
Infiltrate, lymphocyte, submucosa, minimal		3	2				1
Score Expanded Totals		3	2				1
Inflammation, serosa, mixed cell, slight							1
Score Expanded Totals							1
Inflammation, mixed cell, minimal			1				7
slight							7
moderate							1
Score Expanded Totals			1				15
Inflammation, artery, moderate		1					
Score Expanded Totals		1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Iron				Non-supplemented Feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	20 ng/kg/day	p-cresidine 400 ng/kg/day	
GENITOURINARY SYSTEM									
URINARY BLADDER									
Metaplasia, squamous, epithelium, focal.		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
minimal									10
slight									6
moderate									5
Score Expanded Totals									21
Mineralization, focal.									
moderate									1
Score Expanded Totals									1
Necrosis, fibrinoid, artery.									
minimal		1							
Score Expanded Totals		1							
Necrosis, epithelium.									
minimal									1
slight									4
Score Expanded Totals									5
SQUAMOUS CELL CARCINOMA									1
[M]									
TRANSITIONAL CELL CARCINOMA									15
[M]									
(Fatal)									1
Score Expanded Totals									16
TRANSITIONAL CELL PAPILLOMA									10
[B]									

[B] Benign tumor

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
BONE MARROW		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		2	3	16	16	21	20	13
Granulopoiesis, increased.								
minimal		6	3	6	6	2	1	1
slight		1			1	1	1	
moderate						1		
marked							1	
Score Expanded Totals		7	3	6	7	4	3	1
LYMPHOMA [M]			2					
Pigment, macrophage.								
minimal		11	9	5	3		2	5
slight		10	11	1				6
Score Expanded Totals		21	20	6	3		2	11
Increase, fat.								
minimal		1						
Score Expanded Totals		1						
LYMPH NODE - AXILLARY								
LYMPHOMA [M]			(2)					
LYMPH NODE - BRONCHIAL								
Within normal limits		(21)	(21)	(23)	(21)	(18)	(21)	(25)
Fibrin.		8	8	12	12	10	12	10
slight				1				
Score Expanded Totals				1				
Histiocytosis.								
minimal						1	2	2
Score Expanded Totals						1	2	2

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q270117

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 PLACES 2000 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Irrm			Non-supplemented Feed			
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - BRONCHIAL		(21)	(23)	(23)	(21)	(18)	(21)	(25)
Infiltrate, mast cell, increased.								
minimal		3	2	3	5	1	3	5
slight						1	2	
Score Expanded Totals		3	2	3	5	2	5	5
Lymphangiectasis.								
minimal		4	2	2		1		1
Score Expanded Totals		4	2	2		1		1
LYMPHOMA [N].			2					
Pigment, macrophage.								
minimal		5	8	7	2	6	3	8
slight		2	4	1	2	1		
Score Expanded Totals		11	12	8	4	7	3	8
Plasmacytosis.								
minimal		2	1	2	1		1	1
Score Expanded Totals		3	1	2	1		1	1
Eykncosis.								
slight				1				
Score Expanded Totals				1				
LYMPH NODE - MANDIBULAR		(13)	(22)	(25)	(22)	(24)	(25)	(22)
Within normal limits		15	14	16	12	10	12	8
Histiocytosis.								
minimal		8	5	7	8	8	7	9
slight			1	1	1	1	2	3
Score Expanded Totals		8	6	8	9	9	9	12
Hyperplasia.								
minimal						1		

[N] Nalignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICUS70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACER 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - MANDIBULAR		(23)	(22)	(25)	(22)	(24)	(25)	(22)
Hyperplasia.								
Score Expanded Totals						1		
Infiltrate, eosinophil.								
minimal								1
Score Expanded Totals								1
Infiltrate, mast cell, increased.								
minimal		1		2	2	3	4	11
slight						1		
Score Expanded Totals		1		2	2	4	4	11
LYMPHOMA [M].			2	2	2			
Pigment, macrophage.								
minimal							1	
Score Expanded Totals							1	
Plasmacytosis.								
slight						1		
Score Expanded Totals						1		
Extramedullary hematopoiesis.								
minimal					1			
Score Expanded Totals					1			
LYMPH NODE - MESENTERIC		(25)	(24)	(24)	(24)	(24)	(23)	(24)
Within normal limits		23	22	24	24	23	21	24
Histiocytosis.								
slight						1		
Score Expanded Totals						1		

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2006 VI.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
ORGAN/TISSUE Finding	TREATMENT	Feed with Ircm				Non-supplemented Feed		
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-cresidine 400 mg/kg/day
HEMOLYMPHATIC SYSTEM								
Lymph Node - Mesenteric		(25)	(24)	(24)	(24)	(24)	(23)	(24)
Hyperplasia								
minimal						1	1	
Score Expanded Totals						1	1	
Hyperplasia, artery								
moderate		1						
Score Expanded Totals		1						
Infiltrate, neutrophil								
slight						1		
Score Expanded Totals						1		
Inflammation, artery								
moderate		1						
Score Expanded Totals		1						
Lymphangiectasis								
minimal							1	
Score Expanded Totals							1	
Lymphoma (M)			2					
Pigment, macrophage								
minimal		1						
Score Expanded Totals		1						
Plasmacytosis								
minimal						1		
Score Expanded Totals						1		
Lymph Node - Renal			(2)		(1)		(1)	
Within normal limits							1	
Histiocytosis								
minimal					1			
Score Expanded Totals					1			

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IEL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACBS 2000 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron			Non-supplemented Feed		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - RENAL LYMPHOMA [M].			(2) 2		(1) 1		(1) 1
LYMPH NODE - SUBLUMBAR LYMPHOMA [M].			(2) 2				
THYMUS		(23) 16	(25) 19	(25) 18	(25) 20	(25) 18	(24) 17
Within normal limits							(25) 20
Atrophy.							
minimal		1			1		
moderate							1
marked							1
Score Expanded Totals		1			1		1
Cyst.							
minimal		5	2	5	3	2	1
slight		1		1	1	3	
Score Expanded Totals		6	2	6	4	5	1
Hemorrhage, focal.							
slight							1
Score Expanded Totals							1
Hyperplasia, lymphoid.							
minimal			2	2		4	1
slight							1
Score Expanded Totals			2	2		4	1
Hyperplasia, epithelial.							
focal.							
minimal					1		
Score Expanded Totals					1		
LYMPHOMA [M].			2				1

[M] Malignant tumor
 Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICM670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2800 VI.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Food with Iron				Non-supplemented Feed		
	0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-eresidine 400 mg/kg/day	
HEMOLYMPHATIC SYSTEM								
THYROID		(23)	(25)	(25)	(25)	(25)	(24)	(25)
Necrosis, focal.								
slight								1
Score Expanded Totals								1
Necrosis, lymphoid.								
minimal								2
marked				1				
Score Expanded Totals				1				2
SPLEEN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		22	22	24	23	19	22	
Depletion, lymphoid.								
minimal		2				2		
slight								1
Score Expanded Totals		2				2		1
Hyperplasia, lymphoid.								
minimal					1	3	1	
Score Expanded Totals					1	3	1	
Infiltrate, plasma cell.								
minimal					1	1		
slight						1		
Score Expanded Totals					1	1		
LYMPHOMA (N)			2					
Necrosis, lymphoid.								
minimal							1	
Score Expanded Totals							1	
Pigment, macrophage.								
minimal		1	1					
slight								6

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2105 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Iron			Non-supplemented Feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM									
SPLEEN		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Pigment, macrophage.									
moderate								17	
marked								1	
Score Expanded Totals		1	1					24	
Pigment, melanocyte.									
minimal				1		2			
Score Expanded Totals				1		2			
Thrombus, artery.									
minimal		1							
Score Expanded Totals		1							
Extramedullary									
hematopoiesis, increased.						1		6	
minimal								5	
slight								11	
moderate								1	
marked								23	
Score Expanded Totals						1			
Extramedullary									
hematopoiesis, decreased.					1				
minimal					1				
Score Expanded Totals					1				
SYSTEMIC/ANY SITE		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		25	23	25	25	25	24	25	
LYMPHOMA (N)							1		
(fatal)			2						
Score Expanded Totals			2				1		

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 FCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACEB 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented Feed			
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-arsidine 400 ng/kg/day	
DIETARY SYSTEM								
MAMMARY GLAND		(2)	(1)	(1)			(2)	(2)
Within normal limits		2	1	1			2	2
PANCREATIC GLAND		(19)	(22)	(23)	(23)	(22)	(25)	(24)
Within normal limits		11	11	12	11	12	11	13
Atrophy,								
minimal		1		3	2			2
slight		1						1
moderate			1					
Score Expanded Totals		2	1	3	2			3
Betasia,								
minimal		5	4	5	6	4	6	4
slight		1	1	1			1	3
Score Expanded Totals		6	5	6	6	4	7	7
Hyperplasia, acinar,								
minimal		1	2	1	1			
Score Expanded Totals		1	2	1	1			
Hyperplasia, acinar, diffuse,								
minimal						1	3	
slight						1		
Score Expanded Totals						2	3	
Hyperplasia, epithelium, duct,								
minimal			2	2	2	2	5	
slight		1						
Score Expanded Totals		1	2	2	2	2	5	
Inflammation, chronic,								
minimal			1	1	2	4	1	2
Score Expanded Totals			1	1	2	4	3	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 NCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
INTEGUMENTARY SYSTEM								
PREPUTIAL GLAND								
Inflammation, mixed cell.		(19)	(22)	(23)	(23)	(22)	(25)	(24)
minimal		2	3	2	3		1	3
Score Expanded Totals		2	3	2	3		1	3
Metaplasia, squamous, focal.								
minimal								1
Score Expanded Totals								1
SKIN								
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Atrophy, hair follicle.		23	25	25	24	19	22	22
minimal							1	
Score Expanded Totals							1	
CARCINOMA (M), not otherwise specified (NOS), (Fatal)								
Score Expanded Totals								1
Fibrosis, dermis, pinna, focal.								
minimal							1	
Score Expanded Totals							1	
Fibrosis, cartilage, pinna, focal.								
minimal		2			1	3	1	1
Score Expanded Totals		2			1	3	1	1
Granulation tissue, pinna, minimal								
Score Expanded Totals					1			1

[N] N malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day	
INTEGUMENTARY SYSTEM								
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	
Hyperplasia, cartilage, pinna, focal, minimal						1		
Score Expanded Totals						1		
Infiltrate, lymphocyte, fat, minimal						1		
Score Expanded Totals						1		
Inflammation, subcutis, pinna, focal, minimal					1		1	
moderate					1			
Score Expanded Totals					1		1	
Ulcer, pinna, moderate					1			
Score Expanded Totals					1			
NOCTILOSKLETAL SYSTEM								
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		25	24	25	25	25	25	
LYMPHOMA [M]			1					
BONE - STERNUM		(25)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		25	24	25	24	25	25	
Degeneration, cartilage, focal, slight					1			

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding		INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Izm				Non-supplemented Feed		
TREATMENT		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	β-estradiol 400 ng/kg/day
MUSCULOSKELETAL SYSTEM								
BONE - STERNUM		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Degeneration, cartilage, focal.								
Score Expanded Totals					1			
Hyperplasia, cartilage, focal.								
moderate					1			
Score Expanded Totals					1			
LYMPHOMA [M]			1					
Mineralization, focal, marked					1			
Score Expanded Totals					1			
DIAPHRAGM				(1)				
Fibrin.								
moderate				1				
Score Expanded Totals				1				
Bacteria.								
moderate				1				
Score Expanded Totals				1				
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	20	23	2	25	25	24
Degeneration, myofiber, focal.								
minimal			2		21			
Score Expanded Totals			2		21			
Edema.								
minimal					1			1

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICHG70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
MUSCULOSKELETAL SYSTEM								
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Edema.								
slight					1			
Score Expanded Totals					2			1
Inflammation, mixed cell, focal.					1			
minimal					1			
Score Expanded Totals					1			
Mineralization, myofiber, focal.								
minimal			1		11			
Score Expanded Totals			1		11			
Regeneration, myofiber, focal.								
minimal		1	3	1	17			
slight				1	1			
Score Expanded Totals		1	3	2	18			
Regeneration, myofiber, focal, abdominal muscle.								
minimal			1					
Score Expanded Totals			1					
NERVOUS SYSTEM								
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal Limits		17	17	21	18	18	15	20
Cyst, keratinizing.								
slight		1	1					
Score Expanded Totals		1	1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 TCl67D: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Irrm				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-oxazidine 400 ng/kg/day
NERVOUS SYSTEM							
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)
Dilatation, ventricle.							
minimal		2	3	1	4	2	2
slight		1	1	1	1	1	1
moderate							
Score Expanded Totals		3	4	2	5	3	2
Inflammation, artery, meninges.							
slight		1					
Score Expanded Totals		1					
LYMPHOMA [N]			1				
Niperalization, thalamus, focal.							
minimal		3	2	2	2	6	4
Score Expanded Totals		3	2	2	2	6	4
Thrombus, artery, meninges.							
minimal		1					
Score Expanded Totals		1					
NERVE - SCIATIC		(24)	(24)	(25)	(24)	(25)	(24)
Within normal limits		24	24	25	24	25	24
SPINAL CORD		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	25	25	25	24	25
Gliosis, focal.							
slight		1					
Score Expanded Totals		1					

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Ixrm				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
NERVOUS SYSTEM								
SPINAL CORD		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, meninges, focal.								
minimal						1		
Score Expanded Totals						1		
Malacia, focal.								
slight		1						
Score Expanded Totals		1						
RESPIRATORY SYSTEM								
LUNG		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	18	14	20	15	16	12
Crystal, focal.								
minimal		1					1	1
slight		1	1					
moderate								
Score Expanded Totals		2	1			1	1	1
Fibrin, vascular.								
minimal					1		2	
slight						1		4
Score Expanded Totals					1	1	2	4
Fibrosis, pleura, focal.								
minimal				2	3		2	1
slight							1	
Score Expanded Totals				2	3		3	1
Hemorrhage, focal.								
minimal						1	1	
Score Expanded Totals						1	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 IC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
RESPIRATORY SYSTEM								
LUNG		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Histiocytosis, alveolar, focal.								
minimal		1						
slight		2	1					
moderate						1		
Score Expanded Totals		3	1			1		
Hyperplasia, bronchioalveolar, focal.								
minimal				1				
Score Expanded Totals				1				
Hyperplasia, medial, artery, focal.								
slight		1	2					
Score Expanded Totals		1	2					
Hyperplasia, lymphoid tissue.								
minimal		1		4		6	1	1
Score Expanded Totals		1		4		6	1	1
Infiltrate, lymphocyte, slight								1
Score Expanded Totals								1
Inflammation, perivascular.								
minimal		5	2	1				1
slight			1					
Score Expanded Totals		5	3	1				1
Inflammation, pleura, focal.								
minimal		1	1	1	2	3	4	5
slight						1		2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Irm			Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day
RESPIRATORY SYSTEM							
LUNG		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, pleura, focal.							
Score Expanded Totals		1	1	1	2	4	7
Inflammation, pleura, diffuse.							
slight				1			
marked				1			
Score Expanded Totals				2			
Inflammation, mixed cell, focal.							
minimal		1				1	2
Score Expanded Totals		1				1	2
Leucocytosis.							
minimal						1	
marked			1				
Score Expanded Totals			1			1	
Lymphoma (M).							
TRANSITIONAL CELL CARCINOMA (M), metastatic site.							1
Eosinophilia, pleura.							
moderate				2			
Score Expanded Totals				2			
TRACHEA		(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		23	24	24	24	25	25
Inflammation, submucosa, chronic.							
minimal		2					

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	D-cresidine 400 mg/kg/day
RESPIRATORY SYSTEM								
TRACHEA								
Inflammation, submucosa, chronic		(25)	(25)	(24)	(25)	(25)	(25)	(25)
slight								
Score Expanded Totals		2					1	1
Inflammation, mixed cell, minimal					1			
Score Expanded Totals			1		1			
LYMPHOMA [M]								
SOFT TISSUE								
FAT - MEDIASTINAL								
Foreign material present, food				(2)				
slight				1				
moderate				1				
Score Expanded Totals				2				
Inflammation, mixed cell								
slight				1				
marked				1				
Score Expanded Totals				2				
Bacteria								
slight				1				
moderate				1				
Score Expanded Totals				2				

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Irm				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-creosidine 400 ng/kg/day
SPECIAL SENSE ORGANS								
EYE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits				3				2
Atrophy, retina.					1			
minimal					1			
Score Expanded Totals					1			
Cataract.					2			
minimal					1			
slight					1			
moderate					4			
marked					1			
Score Expanded Totals					8			
Degeneration, fiber, lens.					1			
minimal		14	12	10	3	8	11	12
slight		1	3	3	6	2		1
moderate					2		1	
marked					1			
Score Expanded Totals		15	15	13	12	10	12	13
LYMPHOMA (N)			1					
Mineralization, cornea.				3				
minimal		1						
slight			1					
Score Expanded Totals		1	1	3				
Mineralization, lens.								
slight					1			
Score Expanded Totals					1			
Necrosis, single cell, lens.								
minimal							1	
Score Expanded Totals							1	

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
		Feed with Iron				Non-supplemented Feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-creosoline 400 ng/kg/day
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Pyknosis, lens.							
minimal		25	24	22	19	25	23
slight					5	25	23
Score Expanded Totals		25	24	22	24	25	23
PAROTID GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		19	14	20	22	14	18
ADENOMA (B)							
Dilatation, acini.							
minimal		5	7	2	2	4	6
Score Expanded Totals		5	7	2	2	4	6
Hyperplasia, focal.							
minimal						1	
Score Expanded Totals						1	
Infiltrate, lymphocyte.							
minimal		1	5	3	2	9	5
slight		1					2
Score Expanded Totals		2	5	3	2	9	7
Inflammation, mixed cell, focal.							
slight			1				
Score Expanded Totals			1				
Neurcels, focal.							
slight			1				
Score Expanded Totals			1				
LACRIMAL GLAND		(25)	(25)	(25)	(25)	(23)	(25)
Within normal limits			3	2			1

(B) Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	30 ng/kg/day	p-cresidine 400 ng/kg/day
SPECIAL SENSE ORGANS								
LACRIMAL GLAND		(25)	(25)	(25)	(25)	(23)	(23)	(25)
Atrophy, acini.								
minimal		10	5	8	15	11	11	7
slight		11	16	14	9	11	10	7
moderate		2				1		
Score Expanded Totals		23	21	22	24	23	21	14
Infiltrate, lymphocyte.								
minimal		14	5	9	14	6	6	9
slight		11	16	14	9	16	17	15
Score Expanded Totals		25	21	23	23	22	23	24
Metaplasia, focal.								
minimal				3	1	3	2	
slight		1					1	
Score Expanded Totals		1		3	1	3	3	
Vacuolation, acini.								
minimal					1			
Score Expanded Totals					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-6 (CONTD.)
GROUP 1 to 7 (FEMALES)

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-60 ng/kg/day	D-oresidine 400 ng/kg/day
CARDIOVASCULAR SYSTEM								
HEART								
Within normal limits		(25) 21	(25) 24	(25) 23	(25) 23	(25) 23	(25) 23	(25) 25
Degeneration, myocardium, focal.								
slight		1						
Score Expanded Totals		1						
Inflammation, epicardium, focal.								
minimal		1						
slight							1	
moderate								
Score Expanded Totals		1					1	
Inflammation, myocardium, focal.								
minimal							1	
slight		1						
Score Expanded Totals		1					1	
LEMPHOMA (M)			1					
Vacuolation, myocardium, focal.								
minimal				1				
slight						1		
Score Expanded Totals				1		1		
Fibrin, epicardium, focal.								
slight							1	

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-creosidine 400 ng/kg/day
CARDIOVASCULAR SYSTEM							
HEART		(25)	(25)	(25)	(25)	(25)	(25)
Fibrin, epicardium, focal.							
Score Expanded Totals						1	
Inflammation, artery.							
slight						1	
marked		1					
Score Expanded Totals		1				1	
Dysplasia, vascular.							
minimal				1			
Score Expanded Totals				1			
Bacteria, epicardium.							
slight		1			2		
Score Expanded Totals		1			2		
AORTA		(25)	(24)	(25)	(24)	(25)	(25)
Within normal limits		25	24	25	24	24	25
Inflammation, artery.							
moderate						1	
Score Expanded Totals						1	
ARTERY		(1)		(1)		(1)	
Polyarteritis.							
slight						1	
moderate		1		1			
Score Expanded Totals		1		1		1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH970: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day
DIGESTIVE SYSTEM							
SALIVARY GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		12	11	11	13	13	21
Granules, serous, decreased.							
moderate					1		
Score Expanded Totals					1		
Infiltrate, lymphocyte.							
minimal		5	10	14	9	7	4
slight		3	4		1	5	2
Score Expanded Totals		12	14	14	10	12	4
Atrophy, parotid, focal.							
slight		1				1	1
Score Expanded Totals		1				1	1
Mucin, increased.							
slight					1		
Score Expanded Totals					1		
Mucin, decreased, diffuse.							
moderate					1		
Score Expanded Totals					1		
TONGUE		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	25	25	25	24	25
Inflammation, artery.							
moderate		1				1	
Score Expanded Totals		1				1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-creosoline 400 ng/kg/day	
DIGESTIVE SYSTEM								
ESOPHAGUS		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		24	25	24	21	23	24	25
Erosion.								
slight		1						
Score Expanded Totals		1						
Fibrosis, muscularis, focal.								
minimal				1	1		1	
slight						2		
Score Expanded Totals				1	1	2	1	
Hemorrhage.								
slight		1						
Score Expanded Totals		1						
Inflammation, adventitia.								
slight					1			
Score Expanded Totals					1			
Bacteria, adventitia.								
minimal					1			
slight					1			
Score Expanded Totals					2			
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		8	10	7	5	8	9	13
Crystal, limiting ridge.								
minimal						2	2	
slight						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM								
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Crystal, limiting ridge.								
Score Expanded Totals						3	2	
Cyst, glandular stomach.								
minimal		1						
Score Expanded Totals		1						
Cyst, limiting ridge.								
minimal		6	2	2	4	4	4	1
Score Expanded Totals		6	2	2	4	4	4	1
Dilatation, gland, focal.								
minimal		3	11	4	1	1	1	5
slight				1				
Score Expanded Totals		3	11	5	1	1	1	5
Edema, nonglandular.								
minimal					1		1	
slight					1		1	
Score Expanded Totals					1		1	
Erosion, glandular.								
minimal		3	5	3	5	4	5	5
slight		3	2	3	1	2	3	3
moderate					1			
marked					1			
Score Expanded Totals		6	7	6	8	6	8	8
Hyperkeratosis.								
minimal		1	0	6	13	2		1
slight					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLATE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM								
STOMACH								
Hyperkeratosis.		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1	4	6	14	2		1
Infiltrate, lymphocyte.								
minimal		1		1				
Score Expanded Totals		1		1				
Inflammation, glandular,								
mixed cell, focal.								
minimal		6	7	9	5	6	6	6
slight		1				1	1	1
moderate					1			
Score Expanded Totals		7	7	9	6	7	7	7
Inflammation, submucosa,								
nonglandular, chronic.								
minimal		3	1	5	4	3	4	
Score Expanded Totals		3	1	5	4	3	4	
LYMPHOMA [M]								
Metaplasia, limiting ridge.								
minimal		3	1	1	1	7	1	1
slight				1		1		
Score Expanded Totals		3	1	2	1	8	1	1
Vacuolation, epithelium,								
glandular.								
minimal		1			2			
slight					1			

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Food with Iron			Non-supplemented food		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-60 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, epithelium, glandular.							
Score Expanded Totals		1			3		
Vacuolation, epithelium, glandular, focal.							
minimal		2	1	1	1		
slight							
Score Expanded Totals		2	1	2	1		
Edema, glandular, focal.							
minimal				1			1
Score Expanded Totals				1			1
Hyperplasia, epithelium, glandular, focal.							
minimal						1	
Score Expanded Totals						1	
Hyperplasia, epithelium, nonglandular, focal.							
minimal				1	4		1
slight						1	
moderate							1
Score Expanded Totals				1	4	2	2
Inflammation, mixed cell, nonglandular, focal.							
minimal					3		
slight						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

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 PLACES 2000 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-rosastidine 400 mg/kg/day
DIGESTIVE SYSTEM								
STOMACH								
Inflammation, mixed cell, nonglandular, focal, moderate		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals					3	2	1	1
Inflammation, artery, slight				1				
Score Expanded Totals		1		1		1		
Necrosis, glandular, mucosa, focal, minimal						1		
Score Expanded Totals						1		
Ulceration, nonglandular, slight							1	
Score Expanded Totals							1	1
Multinucleated giant cell, limiting ridge, minimal							1	
Score Expanded Totals							1	
DUCHENN		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		25	24	24	24	25	24	25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0290117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron			Non-supplemented feed		
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-560 mg/kg/day	p-cresidine 400 mg/kg/day
DIGESTIVE SYSTEM							
DUODENUM		(25)	(25)	(25)	(24)	(25)	(25)
Dilatation, Brunner's gland, minimal							
Score Expanded Totals						1	1
Hyperplasia, lymphoid tissue, minimal			1	1			
Score Expanded Totals			1	1			
JEJUNUM		(24)	(25)	(25)	(25)	(24)	(25)
Within normal limits		23	23	23	22	22	24
Hyperplasia, lymphoid tissue, minimal				1	1	1	1
slight			2		1	1	
moderate				1			
Score Expanded Totals			2	2	2	2	1
Inflammation, artery, slight		1					
Score Expanded Totals		1					
Necrosis, lymphoid tissue, minimal					1		
Score Expanded Totals					1		
Prominent, Paneth cell, slight						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-50 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM								
JEJUNUM		(24)	(25)	(25)	(25)	(25)	(24)	(25)
Prominent, Paneth cell.								
Score Expanded Totals						1		
ILEUM		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		24	23	21	23	20	21	22
Hyperplasia, lymphoid tissue.								
minimal		1	2	4	1	4	4	3
slight						1		
Score Expanded Totals		1	2	4	1	5	4	3
CECUM		(24)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		17	20	24	22	24	21	22
Hyperplasia, crypt.								
minimal			1					
Score Expanded Totals			1					
Hyperplasia, lymphoid tissue.								
minimal		5	1		3		2	2
slight			3	1		1	1	1
Score Expanded Totals		5	4	1	3	1	3	3
Inflammation, mixed cell.								
minimal		1					1	
Score Expanded Totals		1					1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
DIGESTIVE SYSTEM									
CECUM		(24)	(25)	(25)	(25)	(25)	(25)	(25)	
Necrosis, single cell, epithelium.									
minimal			1						
Score Expanded Totals			1						
Fibrosis, muscularis.									
slight		1							
Score Expanded Totals		1							
Impaction.									
moderate		1							
Score Expanded Totals		1							
Inflammation, transmural.									
minimal		1							
Score Expanded Totals		1							
Necrosis, lymphoid tissue.									
minimal							1		
Score Expanded Totals							1		
Vacuolation, epithelium, vacuoles contain pyknotic material/inflammatory cells.									
slight							1		
Score Expanded Totals							1		
COLON		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		22	23	25	23	25	25	25	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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		INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented feed	
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100->600 ng/kg/day
DIGESTIVE SYSTEM							
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		2	2		1		
Score Expanded Totals		2	2		1		
Inflammation, mucosa, focal.							
minimal					1		
Score Expanded Totals					1		
Inflammation, artery.							
slight		1					
Score Expanded Totals		1					
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	23	23	25	23	24
Hyperplasia, lymphoid tissue.							
minimal			2	1		2	1
slight				1			
Score Expanded Totals			2	2		2	1
MESENTERY					(1)		
Inflammation, chronic active.							
moderate					1		
Score Expanded Totals					1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-50 400 ng/kg/day	p-cresidine 400 ng/kg/day
DIGESTIVE SYSTEM								
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		3	7	8	13	3	12	11
Atrophy, acinar, focal.								
minimal			1	1	1			
marked						1		
Score Expanded Totals			1	1	1	1		
Basophilic, acinar cell,								
focal,								
minimal			1			1		1
slight		1						
Score Expanded Totals		1	1			1		1
Dilatation, duct,								
minimal				1				
Score Expanded Totals				1				
Hyperplasia, islet cell,								
minimal		13	12	7	8	12	7	8
Score Expanded Totals		13	12	7	8	12	7	8
Hypertrophy, acinar, focal.								
minimal		2	2	2	2			
Score Expanded Totals		2	2	2	2			
Infiltrate, lymphocyte,								
minimal		5	9	12	5	15	11	7
Score Expanded Totals		5	9	12	5	15	11	7
Inflammation, chronic								
active, focal.								
slight					1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosidine 400 ng/kg/day
DIGESTIVE SYSTEM								
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, chronic active, focal.					1	1		
Score Expanded Totals					1	1		
Synogen decreased, minimal		3	3	3	1			
Score Expanded Totals		3	3	3	1			
Decrease, islets of Langerhans, minimal		4	1	1	1	1		2
slight			1	1				
Score Expanded Totals		4	2	2	1	1		2
Accessory spleen, slight								1
Score Expanded Totals								1
Edema, interstitial, minimal					1			
Score Expanded Totals					1			
Mitotic figures, increased, focal, slight			1					
Score Expanded Totals			1					
Vacuolation, acinar cell, focal, minimal		1						
moderate		1						

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 FCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-erasidone 400 mg/kg/day
DIGESTIVE SYSTEM								
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, acinar cell, focal.								
Score Expanded Totals		2					1	
ACINAR CELL ADENOCARCINOMA [N]								
HEPATOBIILIARY SYSTEM								
GALL BLADDER		(25)	(22)	(24)	(24)	(24)	(25)	(24)
Within normal limits		23	21	20	22	20	21	23
Hyaline bodies, cytoplasm, epithelium.								
slight						1		
Score Expanded Totals						1		
Infiltrate, lymphocyte.								
minimal			1					
Score Expanded Totals			1					
Inflammation, lamina propria, mixed cell.								
minimal		1		2	2	3	4	
slight				2				1
Score Expanded Totals		1		4	2	3	4	1
Inflammation, artery.								
minimal		1						

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0370117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Food with Iron			Non-supplemented food			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM								
GALL BLADDER		(25)	(22)	(24)	(24)	(24)	(25)	(24)
Inflammation, artery.								
Score Expanded Totals		1						
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Within normal limits		2	1	1	3	2	1	
Angiectasis, focal.								
minimal				1				
slight		1		1		1		
Score Expanded Totals		1		2		1		
Bile stasis.								
minimal				1				5
Score Expanded Totals				1				5
Focus, eosinophilic.								
marked								1
Score Expanded Totals								1
Glycogen, decreased.								
hepatocyte.								
minimal						1	1	
slight					1			
Score Expanded Totals					1	1	1	
Hyperplasia, bile duct.								
minimal				1				1
slight								1
Score Expanded Totals				1			6	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	p-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Hypertrophy, centrilobular.								
minimal		9	15	13	13	6	13	
slight		1	1	3	2	1	1	
Score Expanded Totals		10	16	16	15	7	14	
Hypertrophy, midzonal.								
minimal								1
slight								20
moderate								4
Score Expanded Totals								25
Inclusion, intranuclear, cytoplasmic invagination.								
minimal				2				9
slight								4
Score Expanded Totals				2				13
Inflammation, lymphocyte, perivascular.								
minimal		7	14	11	11	10	6	2
slight		9		1	2	4		1
Score Expanded Totals		16	14	12	13	14	6	3
Inflammation, mixed cell, focal.								
minimal		4	5	6	2	6	2	8
slight						2	1	
Score Expanded Totals		4	5	6	2	8	10	8

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICH570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
LYMPHOMA [N]			1					
Mitotic figures, increased.				1				2
minimal				1				2
Score Expanded Totals				1				2
Necrosis, nontribular.							1	
minimal							1	
Score Expanded Totals							1	
Necrosis, hepatocellular, focal.		2	4	3	1	2	3	5
minimal		2	4	3	1	2	3	5
slight						1	1	
Score Expanded Totals		2	4	3	1	3	4	5
Necrosis, hepatocyte, single cell.								1
minimal								1
Score Expanded Totals				1				1
Necrosis, midzonal.								19
minimal								4
slight								23
Score Expanded Totals								23
Pigment, hepatocellular.								1
minimal								1
slight							1	
Score Expanded Totals							1	1

[N] Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron			Non-supplemented feed		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(24)	(25)	(25)	(24)	(25)
Pigment, Kupffer cell.							
minimal		1		1			3
slight				1			1
Score Expanded Totals		1		2			4
Tension focus.							
minimal		1	1	1	4	3	5
slight		3	1	1	1	1	3
Score Expanded Totals		4	2	2	5	4	8
Vacuolation, hepatocellular, centrilobular.							
minimal					1		
Score Expanded Totals					1		
Vacuolation, hepatocellular, panlobular.							
minimal			1		1		2
slight		1					
Score Expanded Totals		1	1		1		2
Vacuolation, hepatocellular, periportal.							
minimal		13	13	14	5	4	3
slight		2		1			1
Score Expanded Totals		15	13	15	5	4	3

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	β-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Extramedullary hematopoiesis.								
minimal		14	15	18	9	11	13	9
slight				2		3	1	7
Score Expanded Totals		14	15	20	9	14	14	16
HEPATOCELLULAR ADENOMA [B].								
Hyperplasia, nodular, artery.								1
slight		1						
Score Expanded Totals		1						
Inflammation, periportal.								
minimal		1		1			6	3
Score Expanded Totals		1		1			6	3
Inflammation, artery.								
minimal		1						
Score Expanded Totals		1						
Inflammation, granulomatous, focal.								
minimal						1		
Score Expanded Totals						1		
Pigment, bile duct.								
minimal							1	
Score Expanded Totals							1	

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 IC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Irm			Non-supplemented feed			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	β-cyosidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Prominent, sinusoidal, lining cell.								
minimal							1	2
slight				2				
Score Expanded Totals				2			1	2
ENDOCRINE SYSTEM								
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		4	2	4	2	1	5	3
Hyperplasia, cortex, focal.								
minimal		1						
Score Expanded Totals		1						
Hyperplasia, subcapsular cell.								
minimal		20	22	19	21	22	20	21
slight						1		
Score Expanded Totals		20	22	19	21	23	20	21
Hypertrophy, cortex, focal.								
slight					1	1		
Score Expanded Totals					1	1		
Infiltrate, lymphocyte.								
minimal						1		
Score Expanded Totals						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICh670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Sex: Male					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
ENDOCRINE SYSTEM							
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)
Necrosis, focal.		1					
minimal		1					
Score Expanded Totals		1					
Pigment, melanin.		2	7	2	4	6	6
minimal		2	7	2	4	6	6
Score Expanded Totals		2	7	2	4	6	6
Extramedullary hematopoiesis.							
minimal		1					
slight				1			
Score Expanded Totals		1		1			
Bacteria, focal.							
slight		1					
Score Expanded Totals		1					
PARATHYROID		(21)	(21)	(22)	(22)	(22)	(23)
Within normal limits		19	21	19	21	21	21
Hyperplasia, focal.							
minimal		1					
Score Expanded Totals		1					
Cyst.							
moderate		1					
Score Expanded Totals		1					
Ectopic thymus.							
minimal							1

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 DCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Irrm				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-cresidine 400 ng/kg/day
ENDOCRINE SYSTEM								
PARATHYROID								
		(21)	(21)	(22)	(22)	(22)	(22)	(23)
	Ectopic thymus.							
	slight			1	1			1
	moderate						1	
	Score Expanded Totals			1	1		1	2
	Edema.							
	minimal			1				
	Score Expanded Totals			1				
	Infiltrate, lymphocyte.							
	slight			1				
	Score Expanded Totals			1				
PITUITARY								
	Within normal limits	(25)	(25)	(25)	(25)	(25)	(25)	(25)
	24	24	24	25	21	24	25	
	Cyst, pars distalis.							
	minimal	1		1		3	1	
	Score Expanded Totals	1		1		3	1	
	Infiltrate, lymphocyte,							
	pars distalis, focal.							
	minimal					1	1	
	Score Expanded Totals					1	1	
LYMPHOMA [M]								
			1					
THYROID								
	Within normal limits	(25)	(25)	(25)	(24)	(25)	(25)	(25)
	16	23	22	20	22	22	24	

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. 0170117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
ENDOCRINE SYSTEM									
THYROID		(25)	(25)	(25)	(24)	(25)	(25)	(25)	
Cyst, follicular, minimal					1		1	1	
Score Expanded Totals					1		1	1	
Ectopic thymus, minimal				2	1	2			
Score Expanded Totals		3	2	2	1	2	1		
Infiltrate, lymphocyte, minimal		3	2		1	2	1		
Score Expanded Totals		4	2		1	2	1		
Inflammation, artery, slight		1				1			
Score Expanded Totals		5			1	1	1		
Hypertrophy, epithelial, minimal				1					
Score Expanded Totals				1					
Inflammation, focal, minimal		1			1				
Score Expanded Totals		1			1				
Necrosis, focal, minimal		1							
Score Expanded Totals		1							

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 TCl670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
URETER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25	25
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Within normal limits		6	4	11	12	4	2	
Atrophy, tubular, focal.								
minimal								4
slight					1		1	6
moderate								1
marked								1
Score Expanded Totals					1		1	12
Atrophy, tubular, diffuse.								
minimal								1
Score Expanded Totals								1
Eosinophilic tubules.								
minimal		1	7	3	3	6	10	1
slight					1		2	21
moderate					1		1	1
Score Expanded Totals		1	7	3	5	6	13	23
Cast, proteinaceous.								
minimal		13	12	5	5	3	14	3
slight			1		1	3	2	17
Score Expanded Totals		13	13	5	6	11	16	20
Dilatation, tubular.								
minimal					1			2
slight					1			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.3.1
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Dilatation, tubular.								
moderate					1			
Score Expanded Totals					3			3
Fibrosis, interstitial,								
focal.								
minimal								3
Score Expanded Totals								3
Hyalinization, glomeruli.								
minimal				1		1	1	
slight						1		
Score Expanded Totals				1		2	1	
Hydronephrosis.								
minimal							2	
slight							1	
severe								1
Score Expanded Totals							3	1
Hyperplasia, epithelial,								
pelvis.								
minimal						1		
slight								1
Score Expanded Totals						1		1
Infiltrate, lymphocyte.								
minimal		6	7	6	3	9	7	4
slight		5	1	2		1	4	14
moderate								1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH970: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-400 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	
Infiltrate, lymphocytic								
Score Expanded Totals		11	8	8	3	10	19	
LYMPHOMA (N)								
Mineralization, focal								
minimal		1	2	1	2	2	4	
slight							17	
Score Expanded Totals		1	2	1	2	2	21	
Necrosis, epithelium, tubular, focal								
minimal			1			1		
Score Expanded Totals			1			1		
Necrosis, papilla								
minimal				1		1	7	
slight							5	
Score Expanded Totals				1		1	12	
Vacuolation, tubular, epithelium, focal								
minimal					1	1		
slight						1		
Score Expanded Totals					1	2		
Vacuolation, tubular, epithelium, diffuse								
slight					1		2	
Score Expanded Totals					1		2	

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-60 ng/kg/day	β-irradiation 40R ng/kg/day	
GENITOURINARY SYSTEM									
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)	
Increase, mesangial cell.									
minimal				1					
Score Expanded Totals				1					
Cast, granular.									
slight					1				
Score Expanded Totals					1				
Crystal, pelvis.									
slight						1			
Score Expanded Totals						1			
Dilatation, collecting tubule.									
minimal					1			1	
Score Expanded Totals					1			1	
Hyaline droplets, tubular, epithelium.									
minimal					1				
slight					2				
Score Expanded Totals					3				
Inflammation, pelvis.									
slight						1			
Score Expanded Totals						1			
Inflammation, artery.									
moderate		1					1		
Score Expanded Totals		1					1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICh670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosidine 400 ng/kg/day
GENITOURINARY SYSTEM								
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Bacteria, focal.								
minimal		1						
Score Expanded Totals		1						
OVARY		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	19	17	19	15	19	17
Cyst.								
minimal		1	1			1	1	
slight				1	2	1		1
moderate					1			
Score Expanded Totals		1	1	1	3	2	1	1
Dilatation, oviduct.								
minimal							1	
Score Expanded Totals							1	
Hemorrhage, luteal.								
minimal		4	2	6	1	3	3	2
slight			1		1	1		
Score Expanded Totals		4	3	6	2	4	3	2
Hyperplasia, lymphoid.								
minimal						4	1	2
slight		1						1
Score Expanded Totals		1				4	1	3
Hyperplasia, papillary, cystic.								
minimal			1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICH870: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. 0170117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
OVARY		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, papillary, cystic.								
Score Expanded Totals			1					
Hyperplasia, tubulocystoma.								
minimal		1	1	1	2	3		2
slight					1			
Score Expanded Totals		1	1	1	3	3		2
LYMPHOMA [M]								
			1					
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		15	13	14	13	11	15	
Acanthosis, epithelium.								10
minimal								9
slight								19
Score Expanded Totals								
Atypia, epithelium, focal.								7
minimal								2
slight								9
Score Expanded Totals								
Hyperplasia, transitional cell, diffuse.								1
minimal								13
slight								11
moderate								

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Sexes						
		Males				Females		
		Food with Iron				Non-supplemented food		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 500 ng/kg/day
GENITOURINARY SYSTEM								
URINARY BLADDER								
Hyperplasia, transitional cell, diffuse		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals								25
Infiltrate, lymphocyte, submucosa								
minimal		8	9	10	9	11	7	
slight		2	2				2	
moderate			1		1	1		
Score Expanded Totals		10	12	10	10	12	9	
Inflammation, serosa, mixed cell								
minimal								1
Score Expanded Totals								1
Inflammation, mixed cell								
minimal						1		11
slight					1			7
Score Expanded Totals					1	1		18
Inflammation, artery								
minimal		1		1		1		
Score Expanded Totals		1		1		1		
Metaplasia, squamous, epithelium, focal								
minimal								7
slight								14
Score Expanded Totals								21

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.3.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-cresidine 400 mg/kg/day	
GENITOURINARY SYSTEM									
URINARY BLADDER									
Neurosis, epithelium.									
minimal									
slight									
Score Expanded Totals									
TRANSITIONAL CELL CARCINOMA [M].									
Score Expanded Totals									
TRANSITIONAL CELL PAPILLOMA [B].									
Score Expanded Totals									
Congestion.									
moderate									
Score Expanded Totals									
Hemorrhage, submucosa.									
slight									
Score Expanded Totals									
Hyperplasia, artery.									
minimal									
Score Expanded Totals									
Mineralization, serosa.									
focal.									
slight									
Score Expanded Totals									
PAPILLOMA [B], squamous cell.									
Score Expanded Totals									

[B] Benign tumor
 [M] Malignant tumor
 Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICH67D: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. 0290117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosolone 400 ng/kg/day
GENITOURINARY SYSTEM							
UTERUS		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		9	7	12	8	9	14
Atrophy, endometrium.							
minimal		1				1	1
slight					1		1
Score Expanded Totals		1			1	1	2
Hyperplasia, cystic endometrial.							
minimal		11	15	13	15	14	9
slight		4	3		1	1	1
Score Expanded Totals		15	18	13	16	15	10
OSTEOCARCINOMA (M) (fatal)					1		
Score Expanded Totals					1		
VAGINA		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		23	24	23	25	21	21
Edema, muscularis.							
slight				1			
Score Expanded Totals				1			
Hyperplasia, epithelium.							
minimal		1	1	1		2	4
Score Expanded Totals		1	1	1		2	4
Inflammation, artery.							
moderate						1	

(M) Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-oxoindole 400 ng/kg/day
SEMIOURINARY SYSTEM								
VAGINA		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, artery.								
Score Expanded Totals						1		
Mucification.								
slight		1					2	
Score Expanded Totals		1					2	
HEMOLYMPHATIC SYSTEM								
BONE MARROW		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		11	11	14	18	17	17	14
Granulopoiesis, increased.								
minimal		1						
slight		1						1
moderate				2	1	2		
marked		1						
Score Expanded Totals		3		2	1	2		1
LYMPHOMA [N]			1					
Pigment, macrophage.								
minimal		8	11	7		3	2	1
slight		3	2					1
Score Expanded Totals		11	13	7		3	2	2
Increase, fat.								
minimal			1		1			1
Score Expanded Totals			1		1		1	

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 FCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM									
BONE MARROW									
Accumulation, macrophage, focal.		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
slight					1				
Score Expanded Totals					1				
Erythropoiesis, increased.					2		1		
minimal					2		1		
Score Expanded Totals									
Fibrosis, focal.									
minimal		1		1	2	1	1		3
slight				2					1
Score Expanded Totals		1		3	2	1	1		4
Granulopoiesis, decreased.									
minimal						1	1		
Score Expanded Totals						1	1		
Hyperplasia, lymphoid.									
minimal					1				
Score Expanded Totals					1				
Increase, fat.									
minimal					2	1	1		6
slight		1		2		1			
Score Expanded Totals		1		2	2	2	1		6
New bone formation, focal.									
minimal		1		1	2	2	1		3
slight		1		2					
moderate							1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
BONE MARROW		(25)	(25)	(25)	(25)	(25)	(25)	(25)
New bone formation, focal.								
Score Expanded Totals		2		3	2	2	2	3
LYMPH NODE - BRONCHIAL		(22)	(25)	(23)	(19)	(23)	(24)	(20)
Within normal limits		7	7	6	8	8	12	9
Histiocytosis.								
minimal		3	2	2		1		
slight					1	1	2	
moderate								
Score Expanded Totals		3	2	2	2	2	2	
Infiltrate, mast cell, increased.								
minimal		1					1	1
Score Expanded Totals		1					1	1
Lymphangiectasis.								
minimal		2	6	4		5	2	
Score Expanded Totals		2	6	4		5	2	
LYMPHOMA (M).			1					
Pigment, macrophage.								
minimal		9	9	12	1	3	5	10
slight		2	4	2	1	4	2	1
Score Expanded Totals		11	13	14	4	7	7	11
Plasmacytosis.								
minimal		4	5	4	2	5	3	

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICN670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Irrm			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	300-500 ng/kg/day	p-creosole 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - BRONCHIAL		(22)	(25)	(23)	(19)	(23)	(24)	(20)
Plasmacytosis								
slight		1		1		1		
Score Expanded Totals		5	5	5	2	6	3	
Depletion								
minimal					2			
slight						1		
Score Expanded Totals					2	1		
Erythrophagocytosis								
minimal			1					
Score Expanded Totals			1					
Hyperplasia								
minimal		2			1	2	1	
slight							1	
Score Expanded Totals		2			1	2	2	
Infiltrate, neutrophil								
minimal				1	1		1	
Score Expanded Totals				1	1		1	
Necrosis								
minimal					2			
Score Expanded Totals					2			
Plasmacytosis, with Russell bodies								
slight						1		
Score Expanded Totals						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Sexes						
		Feed with Iron				Non-supplemented feed		
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-cresidine 400 mg/kg/day	
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - BRONCHIAL		(22)	(25)	(23)	(19)	(21)	(24)	(20)
Plasmacytosis, Russell bodies.								1
minimal								1
Score Expanded Totals								1
Bacteria.					1			
slight					1			
Score Expanded Totals					1			
LYMPH NODE - MANDIBULAR		(24)	(23)	(25)	(22)	(23)	(24)	(25)
Within normal limits		10	11	12	5	7	8	10
Histiocytosis.								
minimal		6	9	11	10	11	8	12
slight		3				1		
Score Expanded Totals		9	9	11	10	12	8	12
Hyperplasia.								
minimal		1				1	1	
slight								1
Score Expanded Totals		1				1	1	1
Infiltrate, mast cell, increased.								
minimal		1	3	1	3	2	5	2
Score Expanded Totals		1	3	1	3	2	5	2
Pigment, macrophage.								
minimal		2	1	3	2			1
Score Expanded Totals		2	1	3	2			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-eresidine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - MANDIBULAR		(24)	(23)	(25)	(22)	(23)	(24)	(25)
Plasmacytosis								
minimal			1		1	1		2
slight						2		1
Score Expanded Totals			1		1	3		3
Extramedullary hematopoiesis								
minimal				2				1
Score Expanded Totals				2				1
Crystal, macrophage								
minimal		1						
Score Expanded Totals		1						
Depletion								
slight							1	
Score Expanded Totals						1		
Hemorrhage								
slight		1						
moderate								
Score Expanded Totals		1						1
Infiltrate, neutrophil								
minimal					1			1
Score Expanded Totals					1			1
Lymphoidplasia								
minimal		1			1			
Score Expanded Totals		1			1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2002 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Food with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	D-creosol 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - MANDIBULAR		(24)	(23)	(25)	(22)	(23)	(24)	(25)
Neurosis								
minimal					1			
slight					2		2	
Score Expanded Totals					3		2	
LYMPH NODE - MEDIASTINAL		(1)						
Histiocytosis								
minimal		1						
Score Expanded Totals		1						
Hyperplasia								
minimal		1						
Score Expanded Totals		1						
LYMPH NODE - MESENTERIC		(25)	(25)	(25)	(25)	(25)	(24)	(25)
Within normal limits		24	22	22	21	23	19	25
Histiocytosis								
minimal		1						
Score Expanded Totals		1						
Infiltrate, neutrophil.								
slight					1			
Score Expanded Totals					1			
Inflammation, attery.								
moderate				1		1		
Score Expanded Totals				1		1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICM570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-cresidine 400 mg/kg/day
HEMOLYMPHATIC SYSTEM								
LYMPH NODE - MESPENTERIC		(25)	(25)	(25)	(25)	(25)	(24)	(25)
Lymphangiectasis.								
minimal				1				
Score Expanded Totals				1				
Pigment, macrophage.								
minimal			3					
Score Expanded Totals			3					
Aggregation, macrophage.								
minimal			2					
Score Expanded Totals			2					
Depletion.								
minimal					1		3	
slight					1			
Score Expanded Totals					2		3	
Necrosis.								
minimal			1		1	1	4	
Score Expanded Totals			1		1	1	4	
Necrosis, artery.								
slight							1	
Score Expanded Totals							1	
Extramedullary hematopoiesis.								
moderate				1				
Score Expanded Totals				1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - PANCREATIC LYMPHOMA [M].						(11) 1	
THYMUS		(25) 19	(25) 20	(24) 16	(25) 12	(24) 21	(25) 14
Within normal limits							(25) 20
Atrophy.							
minimal					4	1	1
slight	1				2		
moderate					1	1	
marked							
Score Expanded Totals	1				7	2	4
Cyst.							
minimal	2	1	5	2			3
slight	1			1	2		
Score Expanded Totals	3	1	5	3	2		3
Hyperplasia, lymphoid.							
minimal	2	3		2			
slight	1		1				1
Score Expanded Totals	3	3	1	2			1
Hyperplasia, epithelial, focal.							
minimal			1				
Score Expanded Totals			1				
LYMPHOMA [M].		1	1				2

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 IC5678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
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 PLACEB 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-arsidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
THYROID		(25)	(25)	(24)	(25)	(24)	(25)	(25)
Necrosis, lymphoid.								
minimal					1		2	1
slight					3		2	
moderate							1	
marked							5	
Score Expanded Totals					4			1
Accumulation, macrophage, focal.								
slight				1				
Score Expanded Totals				1				
SPLEEN		(25)	(24)	(25)	(25)	(25)	(25)	(25)
Within normal limits		10	12	16	11	17	15	
Depletion, lymphoid.								
minimal		1			1	2	1	
slight		1			2	1	1	
moderate							1	
Score Expanded Totals		2			3	3	2	
Hyperplasia, lymphoid.								
minimal		2		4		1	1	
slight		1						
moderate		1						
Score Expanded Totals		4		4		1	1	
Infiltrate, plasma cell.								
minimal					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02Y0117

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 PLACES 2000 Y1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Food with Irra				Non-supplemented feed	
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	p-rossidine 400 mg/kg/day
HEMOLYMPHATIC SYSTEM							
SPLEEN		(25)	(24)	(25)	(25)	(25)	(25)
Infiltrate, plasma cell.					1		
Score Expanded Totals							
Lymphoma (N)			1			1	
Necrosis, lymphoid.					1	2	
minimal					1		
moderate					1	2	
Score Expanded Totals							
Pigment, macrophage.							
minimal		7	4	3	5	4	1
slight		3	6	4	2		9
moderate							16
Score Expanded Totals		10	10	7	7	4	25
Pigment, melanocyte.							
slight						1	
Score Expanded Totals						1	
Extramedullary hematopoiesis, increased.							
minimal		4	2		4	3	3
slight		2	1		1	3	15
moderate				1		2	9
marked				1			
Score Expanded Totals		6	3	2	5	8	24
Accessory spleen.			1				

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 ICLE70: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Food with Irm			Non-supplemented food			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
SPLLEN		(25)	(24)	(25)	(25)	(25)	(25)	(25)
Congestion.								
minimal				1				
slight					1			
Score Expanded Totals				1	1			
Deformity.								
minimal				1				
Score Expanded Totals				1				
SYSTEMIC/ANY SITE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	24	25	25	25	23	25
LYMPHOMA [M]		1					2	
(fatal)			1					
Score Expanded Totals		1	1				2	
INTEGUMENTARY SYSTEM								
CUTANEOUS GLAND		(10)	(14)	(11)	(10)	(13)	(13)	(16)
Within normal limits		8	10	6	6	7	12	9
Ectasia.								
minimal					2			
slight						1		
Score Expanded Totals					2	1		
Hyperplasia, acinar.								
minimal						1		

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICHS70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. Q270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
INTRELUMENTARY SYSTEM								
CLITORAL GLAND		(10)	(14)	(11)	(10)	(13)	(13)	(16)
Hyperplasia, acinar, Score Expanded Totals						1		
Hyperplasia, acinar, diffuse, slight Score Expanded Totals			1					
Inflammation, mixed cell, minimal slight Score Expanded Totals		2	2	3	2	5	1	7
MAMMARY GLAND		(24)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		22	25	23	24	23	24	25
Hyperplasia, duct, minimal Score Expanded Totals		2		1	1	2	1	
ADENOCARCINOMA (N1, PNI (fatal) onset day=121. Score Expanded Totals				1				
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		14	22	20	18	21	23	19

(N1) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
INTEGUMENTARY SYSTEM								
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Atrophy, hair follicle.				1	1	1		
minimal								
slight								
Score Expanded Totals				1	1	1		
Fibrosis, dermis, pinna, focal.								
minimal		4	1		2		1	1
slight					1		3	
moderate			1					1
Score Expanded Totals		4	2		3		3	2
Fibrosis, cartilage, pinna, focal.								
minimal		4		1	3	1	1	2
slight			1	1				
Score Expanded Totals		4	1	2	3	1	1	2
Hyperplasia, cartilage, pinna, focal.								
minimal								2
Score Expanded Totals								2
Inflammation, subcutis, pinna, focal.				1		1		
minimal		1			1			1
slight								2
moderate			1		1			2
Score Expanded Totals		1	1	1	2	1		5

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Izum			Non-supplemented feed		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-50 ng/kg/day	p-cresidine 400 ng/kg/day
INTEGUMENTARY SYSTEM							
SKIN		(25)	(25)	(25)	(25)	(25)	(25)
Ulcer, pinna,							
minimal		1					
slight							1
moderate					1		
Score Expanded Totals		1			1		1
Hyperplasia, epidermis,							
pinna, focal,							
minimal		1	1		1		
slight		1					
Score Expanded Totals		1	1		1		
Inflammation, epidermis,							
focal,							
slight				1			
Score Expanded Totals				1			
Inflammation, subcutis,							
chronic active,							
slight		1				1	
moderate		1					
marked							
Score Expanded Totals		2				1	
Mineralization, cartilage,							
pinna, focal,							
minimal		2					
slight				1			1
Score Expanded Totals		2		1			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICM670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	β-cresidine 400 ng/kg/day
INTEGUMENTARY SYSTEM								
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Neuritis,								
slight						1		
Score Expanded Totals						1		
Ulcer,								
moderate						1		
Score Expanded Totals						1		
OSTEOSARCOMA [M], PNI onset								
day-172,								
(fatal)		1						
Score Expanded Totals		1						
MUSCULOSKELETAL SYSTEM								
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25	24
OSTEOSARCOMA [M],								1
BONE - STERNUM		(25)	(25)	(25)	(25)	(25)	(25)	(24)
Within normal limits		25	25	25	25	25	24	24
Hyperplasia, cartilage,								
focal,								
slight							1	
Score Expanded Totals							1	

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-creosidine 400 ng/kg/day
MUSCULOSKELETAL SYSTEM								
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	22	24	16	23	25	23
Degeneration, myofiber, focal.								
minimal					5	1		
Score Expanded Totals					5	1		
Edema.								
minimal					1			
Score Expanded Totals					1			
Inflammation, mixed cell, focal.								
minimal			1					
Score Expanded Totals			1					
Mineralization, myofiber, focal.								
minimal					3			
Score Expanded Totals					3			
Regeneration, myofiber, focal.								
minimal		1	3	1	5	1		
slight					1			
Score Expanded Totals		1	3	1	6	1		
Inflammation, mixed cell, abdomen.								
slight								2
moderate					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-360 mg/kg/day	p-cresidine 400 mg/kg/day
MUSCULOSKELETAL SYSTEM								
MUSCLE								
Inflammation, mixed cell, abdomen.		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals					1			2
Inflammation, artery, moderate						1		
Score Expanded Totals						1		
Necrosis, artery, slight						1		
Score Expanded Totals						1		
NERVOUS SYSTEM								
BRAIN								
Within normal limits		(25) 23	(25) 25	(25) 23	(25) 23	(25) 22	(25) 25	(25) 25
Dilatation, ventricle, minimal		2		1	2	3		
Score Expanded Totals		2		1	2	3		
Inflammation, artery, neuroglia, slight		1						
moderate				1				
Score Expanded Totals		1		1				
Gitter cell, focal, minimal		1						
Score Expanded Totals		1						

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH67B: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-50 ng/kg/day	p-cresidine 400 ng/kg/day
NERVOUS SYSTEM								
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, artery, choroid plexus, moderate						1		
Score Expanded Totals						1		
Necrosis, meninges, artery, moderate				1				
Score Expanded Totals				1				
Necrosis, artery, choroid plexus, slight						1		
Score Expanded Totals						1		
NERVE - SCIATIC		(24)	(24)	(24)	(25)	(24)	(24)	(24)
Within normal limits		24	24	24	25	24	24	24
SPINAL CORD		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25	25
RESPIRATORY SYSTEM								
LARYNX						(1)		
Inflammation, mixed cell, slight						1		
Score Expanded Totals						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-60 ng/kg/day	p-cresidine 400 ng/kg/day	
RESPIRATORY SYSTEM								
LUNG		(15)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		10	15	16	12	8	13	15
Crystal, focal.								
minimal				1		1		1
slight						3		
moderate							2	
Score Expanded Totals				1		4	2	1
Fibrosis, pleura, focal.								
minimal		2	1					1
Score Expanded Totals		2	1					1
Hemorrhage, focal.								
minimal			1		1			1
moderate		1						
Score Expanded Totals		1	1		1			1
Histiocytosis, alveolar, focal.								
minimal						1		2
slight				1		2		
moderate							1	
marked							1	
Score Expanded Totals				1		3	2	2
Hyperplasia, bronchioalveolar, focal.								
minimal				1			1	1
slight								1
Score Expanded Totals				1			1	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02P0117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-560 mg/kg/day	p-creosoline 400 mg/kg/day
RESPIRATORY SYSTEM								
LUNG		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Hyperplasia, medial, artery, focal, minimal			1					
Score Expanded Totals			1					
Hyperplasia, lymphoid tissue, minimal		1	1	2	1	4	3	2
slight		1	1			1		
moderate							1	
Score Expanded Totals		3	2	2	1	5	4	2
Inflammation, perivascular, minimal		8	5	7	4	10	6	5
slight		1			1	3	2	1
moderate				1		1	1	
Score Expanded Totals		9	5	8	5	13	9	6
Inflammation, pleura, focal, minimal		1	1		3	1	2	
slight		1			1			
Score Expanded Totals		2	1		4	1	2	
LYMPHOMA (M)		1	1				1	
Bacteria, pleura, moderate					2			
Score Expanded Totals		1			2			

(M) Malignant tumor
 Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICM670: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-560 mg/kg/day	B-cresidine 400 mg/kg/day
RESPIRATORY SYSTEM								
LUNG		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Congestion								
minimal							1	
slight							1	
Score Expanded Totals							2	
Fibrosis, interstitial, focal								
slight							1	
Score Expanded Totals							1	
Hyaline nodules, bronchial, epithelium								
slight						1		
Score Expanded Totals						1		
TRACHEA		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		23	23	24	23	21	24	24
Inflammation, submucosa, chronic								
minimal		1	2	1	1	2	1	1
Score Expanded Totals		1	2	1	1	2	1	1
Inflammation, mixed cell								
minimal		1				2		
Score Expanded Totals		1				2		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Irrm			Non-supplemented feed		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day
SOFT TISSUE							
FAT			(1)				
Necrosis, abdominal, focal, moderate			1				
Score Expanded Totals			1				
FAT - MEDIASTINAL					(3)		
Inflammation, mixed cell, slight					1		
moderate					1		
Score Expanded Totals					2		
Bacteria, moderate					2		
Score Expanded Totals					2		
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		6	5	6	5	5	9
Cataract, minimal					1		
slight					4		1
moderate					8		
Score Expanded Totals					13		1
Regeneration, fiber, lens, minimal		5	2	3	1		2
slight			2	2	1		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Sex: male					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	p- cresidine 400 ng/kg/day
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Degeneration, fiber, lens.					1		
moderate					1		
marked							
Score Expanded Totals		5	4	5	4	3	9
Mineralization, cornea.							
minimal		1		1		1	
slight							
Score Expanded Totals		1		1		1	
Mineralization, lens.							
slight					1		
Score Expanded Totals					1		
Necrosis, single cell, lens.							
minimal					1		
Score Expanded Totals					1		
Dyskeratosis, lens.							
minimal		19	19	17	9	19	21
slight			1	2	5	2	
moderate					1		
Score Expanded Totals		19	20	19	15	19	21
Inflammation, anterior chamber.							
slight						1	
Score Expanded Totals						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented Feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day
SPECIAL SENSE ORGANS								
EYE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, cornea.								
slight							1	
Score Expanded Totals							1	
Ulceration, cornea.								
slight							1	
Score Expanded Totals							1	
BARBERIAN GLAND		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	18	22	18	19	22	22
Dilatation, acini.								
minimal		1			1			
Score Expanded Totals		1			1			
Infiltrate, lymphocyte.								
minimal		6	6	3	6	6	3	3
slight		1						
Score Expanded Totals		7	6	3	6	6	3	3
Inflammation, mixed cell.								
slight			1					
Score Expanded Totals			1					
LACRIMAL GLAND		(25)	(22)	(25)	(20)	(25)	(25)	(25)
Within normal limits		10	10	13	18	9	19	15
Atrophy, acini.								
minimal		5	1		1	2	2	
slight				1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	5- 400 ng/kg/day
SPECIAL SENSE ORGANS								
LACRIMAL GLAND		(25)	(22)	(25)	(24)	(25)	(25)	(25)
Atrophy, acini								
Score Expanded Totals		5	1	1	1	2	2	
Infiltrate, lymphocyte								
minimal		12	9	7	6	12	5	8
slight		1	1	5		2	1	1
Score Expanded Totals		15	10	12	6	14	6	9
Metaplasia, focal								
minimal		1	1			1		
slight								1
Score Expanded Totals		1	1			1		1
Mucin, decreased, diffuse								
slight					1			
Score Expanded Totals					1			
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES								
HEAD								
Within normal limits					(1) 1			
TAIL								
Within normal limits						(1) 1		(1) 1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-6 (CONTD.)

GROUP 8 AND 9

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117r

5-MAR-04 13:28
 Page : 1
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Female	
	Non-supplemented Feed	
TREATMENT	0 ng/kg/day	30 ng/kg/day
CARDIOVASCULAR SYSTEM		
HEART		
Within normal limits	(25) 25	(25) 23
Inflammation, artery, myocardium, marked		1
Score Expanded Totals		1
Inflammation, epicardium, focal, moderate		1
Score Expanded Totals		1
Inflammation, endocardium, focal, moderate		1
Score Expanded Totals		1
AORTA		
Within normal limits	(23) 23	(24) 23
Inflammation, mixed cell, focal, slight		1
Score Expanded Totals		1
ARTERY		
Polyarteritis, moderate		(1) 1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:28
 Page : 2
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
SALIVARY GLAND		
Within normal limits	(25) 10	(25) 13
Infiltrate, lymphocyte, minimal	9	10
slight	5	2
Score Expanded Totals	14	12
LAMPHOMA [M]	1	
TONGUE		
Within normal limits	(25) 25	(25) 25
ESOPHAGUS		
Within normal limits	(25) 21	(25) 20
Fibrosis, muscularis, focal, minimal	1	1
slight	3	3
Score Expanded Totals	4	4
Inflammation, muscularis, focal, slight		1
Score Expanded Totals		1
Degeneration, muscularis, focal, moderate		1
Score Expanded Totals		1

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02V8117E

5-MAR-04 13:28
 Page : 3
 PLMCS 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Female
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
STOMACH		
Within normal limits	(25) 8	(25) 11
Cyst, limiting ridge.		
minimal		1
slight	1	2
Score Expanded Totals		3
Dilatation, gland, focal.	1	3
minimal		4
Score Expanded Totals	2	4
Erosion, glandular.	2	4
minimal		4
slight	4	4
Score Expanded Totals		1
Hyperkeratosis.	4	5
minimal		1
Score Expanded Totals	2	1
Inflammation, glandular, mixed cell, focal.	2	1
minimal		9
slight	1	10
Score Expanded Totals	9	10
Metaplasia, limiting ridge.		
minimal		3
slight	3	1
Score Expanded Totals		1
Vacuolation, epithelium, glandular, focal.	3	1
minimal	1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL570: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0278117E

5-MAR-04 13:38
 Page 4
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 ng/kg/day	30 ng/kg/day
DIGESTIVE SYSTEM		
STOMACH		
Vacuolation, epithelium, glandular, focal.	(25)	(25)
Score Expanded Totals	1	1
Inflammation, artery, marked		1
Score Expanded Totals		1
Inflammation, nonglandular, submucosa, chronic, minimal	5	2
Score Expanded Totals	1	2
Cyst, glandular, minimal	1	1
Score Expanded Totals	1	1
DUODENUM		
Within normal limits	(25)	(25)
Dilatation, Brunner's gland, slight	25	24
Score Expanded Totals		1
JEJUNUM		
Within normal limits	(25)	(25)
Hyperplasia, lymphoid tissue, minimal	24	25
Score Expanded Totals	1	1

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICM670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q270117E

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 Page : 5
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
ILEUM		
Within normal limits	(25) 23	(25) 24
Hyperplasia, lymphoid tissue, minimal	2	1
Score Expanded Totals	2	1
CECUM		
Within normal limits	(25) 21	(25) 24
Hyperplasia, lymphoid tissue, minimal	4	1
Score Expanded Totals	4	1
COLON		
Within normal limits	(25) 23	(25) 24
Hyperplasia, lymphoid tissue, minimal	2	1
Score Expanded Totals	2	1
RECTUM		
Within normal limits	(25) 24	(25) 22
Hyperplasia, lymphoid tissue, minimal	1	3
Score Expanded Totals	1	3
PANCREAS		
Within normal limits	(25) 8	(25) 10

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701177

5-MAR-04 13:28
 Page : 6
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
PANCREAS		
Atrophy, exocrine, focal.		(25)
slight		
Score Expanded Totals	1	(25)
Hyperplasia, islet cell.		
minimal		
Score Expanded Totals	5	9
Infiltrate, lymphocyte.		
minimal		
Score Expanded Totals	14	6
Decrease, islets of Langerhans.		
minimal		
Score Expanded Totals	2	4
Pigment, macrophage, focal.		
slight		
Score Expanded Totals	1	4
Inflammation, focal, chronic.		
slight		
Score Expanded Totals	1	
Hemorrhage, focal.		
minimal		
Score Expanded Totals	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICD670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 Page : 7
 DLACS 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEPATOBIILIARY SYSTEM		
GALL BLADDER		
Within normal limits	(25) 24	(25) 24
Inflammation, mixed cell, lamina propria.		
minimal	1	1
slight		1
Score Expanded Totals	1	1
LIVER		
Within normal limits	(25) 1	(25) 1
Angiectasis, focal.		
minimal		2
Score Expanded Totals		2
Hypertrophy, centrilobular.		
minimal	7	5
Score Expanded Totals	7	5
Hypertrophy, midzonal.		
minimal	1	
Score Expanded Totals	1	
Inflammation, lymphocyte, perivascular.		
minimal	10	8
slight	3	3
Score Expanded Totals	13	11
Inflammation, mixed cell, focal.		
minimal	8	11
Score Expanded Totals	8	11

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117C

5-MAR-04 13:28
 Page : 8
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEPATOBIILIARY SYSTEM		
LIVER	(25)	(25)
Necrosis, hepatocellular, focal.		
minimal	4	7
slight	1	
Score Expanded Totals	5	7
Tumors focus.		
minimal	5	1
slight	3	2
Score Expanded Totals	8	3
Vacuolation, hepatocellular, periportal.		
minimal	4	3
slight	1	
Score Expanded Totals	5	3
Extraschillary hematopoiesis.		
minimal	14	16
slight		1
Score Expanded Totals	14	17
Inclusion, intranuclear, cytoplasmic invagination.		
minimal	2	
Score Expanded Totals	2	
Multinucleated giant cell, focal.		
minimal		1
Score Expanded Totals		1
Inflammation, periportal.		
minimal		1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

5-MAR-04 13:28
 Page : 5
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEPATOCHOLELITARY SYSTEM		
LIVER		
Glycogen, decreased.	(25)	(25)
slight		1
Score Expanded Totals		1
ENDOCRINE SYSTEM		
ADRENAL		
Within normal limits	(25)	(25)
Hyperplasia, subcapsular cell.	4	5
minimal	20	20
slight	1	1
Score Expanded Totals	21	20
Hyperplasia, medulla, focal.	1	1
slight	1	1
Score Expanded Totals	1	1
PARATHYROID		
Within normal limits	(22)	(21)
Cyst.	20	21
minimal	1	1
Score Expanded Totals	1	1
Ectopic thymus.	1	1
minimal	1	1
Score Expanded Totals	1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 Page: 10
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 mg/kg/day	30 mg/kg/day
ENDOCRINE SYSTEM			
PITUITARY		(25)	(25)
Within normal limits		25	25
THYROID		(25)	(25)
Within normal limits		23	21
Ectopic thymus.			
minimal		1	2
slight		1	
Score Expanded Totals		2	2
Infiltrate, lymphocyte.			
minimal			1
Score Expanded Totals			1
Cyst.			
minimal		1	
Score Expanded Totals		1	
Inflammation, artery.			
slight			1
Score Expanded Totals			1
GENITOURINARY SYSTEM			
CERVIX		(25)	(25)
Within normal limits		25	25
KIDNEY		(25)	(25)
Within normal limits		5	10

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701172

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Food	
		0 mg/kg/day	30 mg/kg/day
GENITOURINARY SYSTEM			
KIDNEY		(25)	(25)
Eosophilic tubules.			
minimal		4	4
Score Expanded Totals		4	4
Cast, proteinaceous.			
minimal		9	9
Score Expanded Totals		9	9
Hyalinization, glomeruli.			
minimal			1
Score Expanded Totals			1
Infiltrate, lymphocytic.			
minimal		10	7
slight		1	1
Score Expanded Totals		11	8
Mineralization, focal.			
minimal		2	
Score Expanded Totals		2	
Vacuolation, tubular, epithelium, focal.			
minimal			1
Score Expanded Totals			1
Dilatation, pelvis.			
minimal			1
Score Expanded Totals			1
Atrophy, tubular, focal.			
minimal			1
Score Expanded Totals			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICI670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:28
 Page: 12
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 mg/kg/day	30 mg/kg/day
GENITOURINARY SYSTEM			
OVARY		(25)	(25)
Within normal limits		10	14
Cyst.			
minimal		1	1
slight		2	1
Score Expanded Totals		3	2
Hemorrhage, uterine.			
minimal		0	3
slight			2
Score Expanded Totals		0	5
Hyperplasia, lymphoid.			
minimal		3	1
slight		2	
Score Expanded Totals		5	1
Hyperplasia, tubular/stratified.			
minimal		3	4
slight		1	
Score Expanded Totals		4	4
URINARY BLADDER		(25)	(25)
Within normal limits		14	9
Infiltrate, lymphocyte, submucosa.			
minimal		11	12
slight			2
Score Expanded Totals		11	14

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117c

5-NOV-04 13:28
 Page: 13
 PLACES 2005 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
GENITOURINARY SYSTEM		
URINARY BLADDER		
Inflammation, artery, moderate		(25)
Score Expanded Totals		(25)
Mineralization, vascular, focal, slight		1
Score Expanded Totals		1
UTERUS		
Within normal limits	(25)	(25)
Atrophy, endometrium, minimal	15	11
slight	1	1
Score Expanded Totals	1	2
Hyperplasia, cystic endometrial, minimal	7	10
slight	2	2
Score Expanded Totals	9	12
Fibrosis, endometrium, minimal		1
Score Expanded Totals		1
VAGINA		
Within normal limits	(25)	(25)
Hyperplasia, epithelium, minimal	23	22
	2	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117f

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 ng/kg/day	30 ng/kg/day
GENITOURINARY SYSTEM			
VAGINA			
Hyperplasia, epithelium. Score Expanded Totals		(25) 2	(25) 2
Inflammation, artery. moderate Score Expanded Totals			1 1
Mucification. slight Score Expanded Totals			1 1
HEMOLYMPHATIC SYSTEM			
BONE MARROW			
Within normal limits		(25) 21	(25) 21
Granulopoiesis, increased. minimal slight moderate Score Expanded Totals		1 1 2	1 1 1
Fibrosis, focal. minimal slight Score Expanded Totals		2 2	1 1
Decrease, fat. minimal slight		2 2	1 1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.2
 ICH675: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Males	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
BONE MARROW	(25)	(25)
Decrease, fat.		
Score Expanded Totals	2	2
New bone formation, focal.		
minimal	2	1
slight		1
Score Expanded Totals	2	2
LYMPH NODE - BRONCHIAL	(22)	(21)
Within normal limits	13	11
Histiocytosis.		
minimal	1	1
Score Expanded Totals	1	1
Infiltrate, mast cell, increased.		
minimal	3	2
Score Expanded Totals	3	2
Lymphangiectasia.		
minimal	2	2
slight		1
Score Expanded Totals	2	3
Pigment, macrophage.		
minimal	8	2
Score Expanded Totals	8	2
Plasmacytosis.		
minimal	1	3
slight		1

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice.
 Study no. 0270117f

5-NAR-04 13:28
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	TREATMENT	Non-supplemented Food
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
LYMPH NODE - BRONCHIAL	(22)	(21)
Plasmacytosis.		
Score Expanded Totals	1	4
Infiltrate, neutrophil.		
slight		1
Score Expanded Totals		1
LYMPH NODE - MANDIBULAR	(24)	(24)
Within normal limits	8	11
Histiocytosis.		
minimal	10	4
Score Expanded Totals	10	4
Infiltrate, mast cell, increased.		
minimal	4	7
moderate	1	
Score Expanded Totals	5	7
Plasmacytosis.		
minimal	1	1
slight		1
Score Expanded Totals	1	2
Infiltrate, neutrophil.		
minimal	1	
Score Expanded Totals	1	
LYMPH NODE - MESENTERIC	(25)	(25)
Within normal limits	24	23

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117c

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM			
LYMPH NODE - MESENTERIC		(25)	(25)
Inflammation, artery, marked			1
Score Expanded Totals			1
Necrosis, slight			1
Score Expanded Totals			1
LYMPHOMA (M)		1	
THYMUS		(25)	(25)
Within normal limits		20	18
Atrophy, minimal			1
moderate			1
Score Expanded Totals			2
Cyst, minimal		3	5
slight			1
Score Expanded Totals		3	6
Hyperplasia, lymphoid, minimal		2	
Score Expanded Totals		2	
Necrosis, lymphoid, slight			1
Score Expanded Totals			1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
THYMUS	(25)	(25)
Inflammation, artery.		
slight		1
Score Expanded Totals		1
SPLEEN	(25)	(25)
Within normal limits	24	20
Depletion, lymphoid.		
moderate		1
Score Expanded Totals		1
Hyperplasia, lymphoid.		
minimal		2
Score Expanded Totals		2
Pigment, macrophage.		
minimal	1	
Score Expanded Totals	1	
Extramedullary hematopoiesis, increased.		
minimal		1
slight		1
Score Expanded Totals		2
Necrosis, lymphoid.		
slight		1
Score Expanded Totals		1
SYSTEMIC/ANY SITE	(25)	(25)
Within normal limits	24	25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701172

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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
SYSTEMIC/ANY SITE LYMPHOMA [N].	(25) 1	(25)
INTEGUMENTARY SYSTEM		
CLITORAL GLAND		
Within normal limits	(16) 8	(11) 8
Ectasia.		
minimal	1	2
Score Expanded Totals	1	2
Inflammation, mixed cell.		
minimal	7	4
Score Expanded Totals	7	4
MAMMARY GLAND		
Within normal limits	(25) 23	(25) 23
Hyperplasia, duct.		
minimal	1	1
Score Expanded Totals	1	1
Fibrosis, periductular.		
minimal	1	
Score Expanded Totals	1	
Dilatation, duct.		
minimal		1
Score Expanded Totals		1

[N] N malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 IC6570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117C

5-MAR-04 13:28
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 PLACES 2000 VJ.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Food
	0 mg/kg/day	30 mg/kg/day
INTEGUMENTARY SYSTEM		
SKIN		
Within normal limits	(25)	(25)
Fibrosis, dermis, pinna, focal.	16	17
minimal	1	1
slight	4	4
moderate	1	1
Score Expanded Totals	5	6
Fibrosis, cartilage, pinna, focal.		
minimal	2	1
slight	1	
Score Expanded Totals	3	1
Inflammation, subcutis, pinna, focal.		
minimal	1	
slight	3	2
moderate	2	3
Score Expanded Totals	6	5
Hyperplasia, epidermis, pinna, focal.		
minimal	3	2
slight	3	1
Score Expanded Totals	6	3
Mineralization, cartilage, pinna, focal.		
slight	1	
Score Expanded Totals	1	
Ulceration, pinna.		
minimal	1	1
slight	2	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
INTEGUMENTARY SYSTEM		
SKIN		
Ulceration, pinna.	(25)	(25)
Score Expanded Totals		
Inflammation, subcutis, focal.	3	2
minimal		
Score Expanded Totals	1	1
Inflammation, epidermis, pinna, focal.	1	1
minimal		
slight		1
Score Expanded Totals	1	1
Necrosis, pinna, focal.	1	1
minimal		
Score Expanded Totals		1
Hyperplasia, cartilage, pinna, focal.		1
minimal		
Score Expanded Totals		1
Granuloma.		1
minimal		
Score Expanded Totals		1
Edema, subcutis, pinna, focal.		1
moderate		
Score Expanded Totals		1
Cyst, epidermal, inclusion.		1
minimal		
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117F

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 PLACES 2800 V1.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Food	
		0 mg/kg/day	30 mg/kg/day
MUSCULOSKELETAL SYSTEM			
BONE - FEMUR/TIBIA		(25)	(25)
Within normal limits		25	25
BONE - STERNUM		(25)	(25)
Within normal limits		25	25
BONE - VERTEBRAE		(1)	
Deformity,			
slight		1	
Score Expanded Totals		1	
MUSCLE		(25)	(25)
Within normal limits		25	23
Regeneration, myofiber, focal,			1
minimal			1
Score Expanded Totals			1
Inflammation, artery,			1
moderate			1
Score Expanded Totals			1
NERVOUS SYSTEM			
BRAIN		(25)	(25)
Within normal limits		24	22
Dilatation, ventricles,		1	1
minimal			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICM678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
NERVOUS SYSTEM		
BRAIN	(25)	(25)
Dilatation, ventricle, slight		1
Score Expanded Totals	1	2
Inflammation, artery, meninges, moderate		1
Score Expanded Totals		1
NERVE - SCIATIC	(25)	(23)
Within normal limits	25	22
Degeneration, slight		1
Score Expanded Totals		1
SPINAL CORD	(25)	(25)
Within normal limits	23	24
Nalacia, focal, marked	1	
Score Expanded Totals	1	
Gliosis, focal, moderate	1	
Score Expanded Totals	1	
Cyst, keratinizing, minimal	1	
Score Expanded Totals	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117f

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 ug/kg/day	30 ug/kg/day
NERVOUS SYSTEM		
SPINAL CORD		
Inflammation, artery, meninges.	(25)	(25)
slight		1
Score Expanded Totals		1
RESPIRATORY SYSTEM		
LUNG		
Within normal limits	(25)	(25)
Crystal, focal.	10	12
minimal		
slight	3	3
Score Expanded Totals	4	3
Histiocytosis, alveolar, focal.		
minimal	2	
slight	2	2
moderate		2
Score Expanded Totals	4	4
Hyperplasia, lymphoid tissue.		
minimal	2	2
slight	1	
Score Expanded Totals	3	2
Inflammation, perivascular.		
minimal	12	7
slight	1	2
Score Expanded Totals	13	9

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117F

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
RESPIRATORY SYSTEM		
LINE	(25)	(25)
Inflammation, mixed cell, focal. minimal	1	2
Score Expanded Totals	1	2
Hyperplasia, artery, medial, focal. minimal	1	
slight	1	1
Score Expanded Totals	2	1
Hyperplasia, pneumocyte Type II, focal. minimal	1	1
slight		2
Score Expanded Totals	1	3
Granuloma, minimal	1	
Score Expanded Totals	1	
Bacteria, pleura. slight		1
Score Expanded Totals		1
Necrosis, focal. moderate		1
Score Expanded Totals		1
Inflammation, pleura. marked		1
Score Expanded Totals		1
Fibrosis, focal. minimal		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 PLACES 2000 VI.080

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Females
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
RESPIRATORY SYSTEM		
LUNG		
Fibrosis, focal.	(25)	(25)
Score Expanded Totals		1
Fibrin, vascular.		1
slight		1
Score Expanded Totals		1
Fibrin, focal.		1
minimal		1
Score Expanded Totals		1
TRACHEA		
Within normal limits	(25)	(25)
Inflammation, submucosa, chronic.	25	22
minimal		3
Score Expanded Totals		3
SPECIAL SENSE ORGANS		
EYE		
Within normal limits	(25)	(25)
Degeneration, fiber, lens.	4	3
minimal		6
Score Expanded Totals	5	6
Dyskinesis, lens.		6
minimal	20	22
slight	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICM670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DERMAL SENSE ORGANS		
EYE		
Dyskeratosis, Lens.	(25)	(25)
Score Expanded Totals	21	22
PAROTID GLAND		
Within normal limits	(25)	(25)
Dilatation, acini.	19	20
minimal	3	3
Score Expanded Totals	3	3
Infiltrate, lymphocyte.	4	3
minimal	4	3
Score Expanded Totals	4	3
LACRIMAL GLAND		
Within normal limits	(22)	(23)
Atrophy, acini.	8	13
minimal	2	3
slight	3	1
Score Expanded Totals	5	4
Infiltrate, lymphocyte.	7	8
minimal	5	2
slight	12	10
Score Expanded Totals	1	1
Metaplasia, focal.		
minimal		
slight		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117C

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 PLACES 2800 V3.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
SPECIAL SENSE ORGANS		
LACRIMAL GLAND		
Metaplasia, focal	(22)	(23)
Score Expanded Totals	1	1
Necrosis, duct,		
minimal	1	
Score Expanded Totals	1	
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES		
MEDIASTINUM		
Inflammation, chronic active, around esophagus with bacteria,		(1)
marked		1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

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APPENDIX-6 (CONTD.)

WILD TYPE MICE

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Table 3.2.3
 ICH507:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117w

5-MAR-04 13:35
 Page : 1
 PLACSE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
CARDIOVASCULAR SYSTEM							
HEART		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, myocardium, focal.							
minimal		2	4	3			
Score Expanded Totals		2	4	3			
Proliferation, myxoid, mitral valve.							
minimal			2	1			
Score Expanded Totals			2	1			
Mineralization, myocardium, focal.							
minimal					2		
Score Expanded Totals					2		
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Inflammation, myocardium, focal.							
minimal		1					
Score Expanded Totals		1					
Inflammation, epicardium.							
minimal					1		
Score Expanded Totals					1		
Atrophy, myocardium.							
minimal				1			
Score Expanded Totals				1			
Within normal limits		22	20	20	22	25	24
AORTA		(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		25	25	24	25	25	25

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117W

5-MAR-04 13:35
 Page: 2
 PLACES 2006 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
	0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day	
CARDIOVASCULAR SYSTEM							
ARTERY							
Polyarteritis, moderate							(1)
Score Expanded Totals							1
DIGESTIVE SYSTEM							
SALIVARY GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Mineralization, duct, minimal		1					
Score Expanded Totals		1					
Metaplasia, squamous, duct, focal, minimal		1					
Score Expanded Totals		1					
Infiltrate, lymphocyte, minimal		14	12	13	13	13	14
slight		4	4	6	2	1	4
Score Expanded Totals		18	16	19	15	14	18
Hyperplasia, ductular, focal, minimal		1					
Score Expanded Totals		1					
Dilatation, duct, minimal			1				
Score Expanded Totals			1				
Atrophy, acini, focal, minimal							1
Score Expanded Totals							1
Within normal limits		7	8	6	10	11	6

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL607: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
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 Page : 3
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 6 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
DIGESTIVE SYSTEM							
TONGUE							
NAST CELL TUMOR [B].							
Inflammation, artery.							
slight							
Score Expanded Totals							
1							
Inflammation, focal, chronic.							
minimal							
Score Expanded Totals							
1							
Within normal limits							
24							
25							
24							
25							
25							
24							
ESOPHAGUS							
Regeneration, myofiber,							
muscularis.							
minimal							
Score Expanded Totals							
1							
Hyperkeratosis.							
minimal							
slight							
Score Expanded Totals							
2							
Within normal limits							
25							
23							
25							
25							
25							
24							
STOMACH							
Ulceration, nonglandular.							
minimal							
moderate							
Score Expanded Totals							
1							
1							
1							
Vacuolation, epithelium,							
glandular, focal.							
minimal							
2							
2							
1							
1							

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117W

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 Page : 4
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, epithelium, glandular, focal.			2	2		1	1
Score Expanded Totals							
Metaplasia, limiting ridge.							
minimal		1	2	1	1		2
slight					1		
Score Expanded Totals		1	2	1	2		2
Inflammation, striary.							
moderate							1
Score Expanded Totals							1
Inflammation, mixed cell, nonglandular, focal.							
moderate							1
Score Expanded Totals							1
Inflammation, submucosa, nonglandular, chronic.							
minimal		2	1	2	5	8	4
slight				1			1
Score Expanded Totals		2	1	3	5	8	5
Inflammation, glandular, mixed cell, focal.							
minimal		4	6	10	8	3	4
slight				1	1		1
marked			1				
Score Expanded Totals		4	7	11	9	3	5
Infiltrate, lymphocyte.							
minimal							1
Score Expanded Totals							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
 Page : 5
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
DIGESTIVE SYSTEM							
STOMACH							
Hyperplasia, glandular, focal.	(25)	(25)	(25)	(25)	(25)	(25)	
minimal					1		
slight				1			
moderate							
Score Expanded Totals		1		1	1		
Hyperplasia, epithelium, nonglandular, focal.							
minimal						3	
slight						1	
Score Expanded Totals						4	
Hyperkeratosis.							
minimal				3	1	14	
Score Expanded Totals				3	1	14	
Foreign material present, hair, nonglandular.							
minimal						1	
Score Expanded Totals						1	
Erosion, glandular.							
minimal	4	2	2	2	3	5	
slight	1		1	1		1	
Score Expanded Totals	5	2	3	3	3	6	
Edema, submucosa, nonglandular.							
slight			1				
Score Expanded Totals			1				
Edema, glandular.							
minimal						1	
Score Expanded Totals						1	
Dilatation, gland, focal.							
minimal		2	3	6	1	2	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
DIGESTIVE SYSTEM							
STOMACH							
Dilatation, gland, focal, slight		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals			2	4	6	1	2
Cyst, limiting ridge, minimal		2	3	2	3	2	4
Score Expanded Totals		2	3	2	3	2	4
Cyst, glandular, minimal			2		1		
Score Expanded Totals			2		1		
Within normal limits		15	13	11	6	12	3
DUODENUM							
Hyperplasia, lymphoid tissue, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals							1
Within normal limits		25	25	25	25	25	24
JEJUNUM							
Hyperplasia, lymphoid tissue, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1	1				
Within normal limits		1	1	25	25	25	25
ILEUM							
Inflammation, mixed cell, focal, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals				1			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL687: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 300 ng/kg/day
DIGESTIVE SYSTEM							
ILEUM		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		2	2		1		
Score Expanded Totals		2	2		1		
Within normal limits		23	23	24	24	25	25
CECUM		(25)	(25)	(25)	(25)	(25)	(25)
Necrosis, single cell.							
epithelium.							
minimal				1			
Score Expanded Totals				1			
Inflammation, mixed cell, fecal.							
slight				1			
Score Expanded Totals				1			
Infiltrate, lymphocyte.							
minimal			1				
Score Expanded Totals			1				
Hyperplasia, lymphoid tissue.							
slight		1					
Score Expanded Totals		1					
Edema, submucosa.							
minimal			2				
Score Expanded Totals			2				
Within normal limits		24	23	23	25	25	25
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Lymphangiectasis.							
minimal			1				
Score Expanded Totals			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027D117W

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
DIGESTIVE SYSTEM							
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		1	2				1
Score Expanded Totals		1	2				1
Edema, submucosa.							
minimal			1				
Score Expanded Totals			1				
Within normal limits		24	22	25	25	25	24
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal					2	1	2
slight							1
Score Expanded Totals					2	1	3
Within normal limits		25	25	25	23	24	22
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)
Decrease, islets of Langerhans.							
minimal					2	4	1
slight			1				
Score Expanded Totals			1		2	4	1
Synogen decreased.							
minimal			4	3		1	
Score Expanded Totals			4	3		1	
Inflammation, mixed cell, focal.							
minimal							1
Score Expanded Totals							1
Infiltrate, lymphocyte.							
minimal		9	12	13	15	13	12
slight		1		1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL60T:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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 PLACES 2808 v1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
DIGESTIVE SYSTEM							
CANCERAS		(25)	(25)	(25)	(25)	(25)	(25)
Infiltrate, lymphocyte.							
Score Expanded Totals		10	12	14	16	13	12
Hypertrophy, vacuolar, focal.							
slight					1	1	
moderate		1					
Score Expanded Totals		1			1	1	
Hypertrophy, acinar, focal.							
minimal		3		2			1
slight							1
Score Expanded Totals		3		2			2
Hyperplasia, islet cell.							
minimal		10	12	10	15	11	12
slight		1	1				
Score Expanded Totals		11	13	14	15	11	12
Basophilic, acinar cell, focal.							
minimal		1	2	1			
Score Expanded Totals		1	2	1			
Atrophy, acinar, focal.							
minimal			1		2	1	1
slight							1
Score Expanded Totals			1		2	1	2
Within normal limits		9	4	5	3	5	9

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEPATORILIARY SYSTEM							
GALL BLADDER		(25)	(23)	(24)	(25)	(25)	(25)
Inflammation, mixed cell, Lamina propria.							
minimal		2	5	3	4	6	5
slight		1		1	4	1	1
Score Expanded Totals		3	5	4	4	7	6
Edema, Lamina propria.							
minimal		1		1			
slight				1			
Score Expanded Totals		1		2			
Within normal limits		21	18	19	21	18	19
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Extramedullary hematopoiesis.							
minimal		11	15	14	15	12	18
slight		1			1	2	
Score Expanded Totals		12	15	14	16	14	18
Vacuolation, hepatocellular, periportal.							
minimal		4	3		16	5	10
slight		2			8		
Score Expanded Totals		6	3		24	5	10
Vacuolation, hepatocellular, perilobular.							
minimal		1	4	2			
slight		1		1			
moderate							
marked		2					
Score Expanded Totals		4	4	3			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, hepatocellular, centrilobular.							
minimal			5	6			
slight		4	4	7			
moderate		3	2	5			
marked		2	2	1			
Score Expanded Totals		15	14	19			
Vacuolation, hepatocellular.							
minimal			1				
Score Expanded Totals			1				
Tension focus.							
minimal			1	2		1	1
slight			2		1		
Score Expanded Totals			3	2	2	1	1
Necrosis, hepatocellular, focal.							
minimal		3	9	2	6	12	7
slight						3	1
moderate					1		
Score Expanded Totals		3	9	2	7	15	8
Karyomegaly, hepatocellular, centrilobular.							
minimal		1	1	3			
Score Expanded Totals		1	1	3			
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Inflammation, mixed cell, focal.							
minimal		2	9	6	11	15	10
slight						2	

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q270117w

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 PLACES 2000 V1.000

ORGAN/TISSUE Finding		INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		TREATMENT	Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEPATOBIILIARY SYSTEM							
LIVER							
Inflammation, mixed cell, focal.		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		2	9	6	11	17	10
Inflammation, periportal.							
minimal						1	1
Score Expanded Totals						1	1
Inflammation, lymphocyte, perivascular.							
minimal		4	5	8	4	2	3
slight		2	5	2	3	2	
Score Expanded Totals		6	11	10	5	10	3
Inclusion, intranuclear, hyaline.							
minimal				1			
Score Expanded Totals				1			
Inclusion, intranuclear, invaginated cytoplasm.							
minimal				1			
Score Expanded Totals				1			
Inclusion, intranuclear, cytoplasmic invagination.							
minimal		4	1	2			
Score Expanded Totals		4	1	2			
Inclusion, intranuclear, cytoplasmic invagination.							
minimal				1			
Score Expanded Totals				1			
Hypertrophy, hepatocellular, centrilobular.							
minimal		22	22	22	7	2	2
slight		2		3			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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 PLACES 2000 V1.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 ng/kg/day	0 ng/kg/day	200 ng/kg/day	0 ng/kg/day	0 ng/kg/day	300 ng/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Hypertrophy, hepatocellular, centrilobular.							
Score Expanded Totals		24	22	25	7	2	10
Hyperplasia, nodular, artery, moderate							1
Score Expanded Totals							1
HISTIOCYTIC SARCOMA [M]					1		
Focus, clear cell, slight			1				
Score Expanded Totals			1				
Dilatation, vascular, minimal			1				
Score Expanded Totals			1				
Within normal limits						3	4
ENDOCRINE SYSTEM							
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)
Pigment, melanin, minimal					5	6	3
Score Expanded Totals					9	6	3
Hypertrophy, cortex, focal, minimal		2		1			
slight			1				
moderate				1			
Score Expanded Totals		2	1	2			
Hyperplasia, subcapsular cell, minimal		6	5	6	22	22	23

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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 PLACES 2600 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 300 ng/kg/day
ENDOCRINE SYSTEM							
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, subcapsular cell. Score Expanded Totals		6	5	6	22	22	23
Hyperplasia, medulla, diffuse. slight Score Expanded Totals			1 1				
Dilatation, vein. minimal Score Expanded Totals				1 1			
Atrophy, cortex. minimal Score Expanded Totals			1 1				
Within normal limits		18	18	17	2	3	2
PARATHYROID		(22)	(23)	(23)	(23)	(24)	(25)
Hyperplasia, focal. minimal Score Expanded Totals							2 2
Ectopic thymus. minimal slight Score Expanded Totals						1 1 2	1 1 1
Cyst. minimal slight Score Expanded Totals				1 1 2	1 1 2	1 1 1	1 1 1
Within normal limits		22	23	22	23	21	21

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
	0 ng/kg/day	0 ng/kg/day	200 ng/kg/day	0 ng/kg/day	0 ng/kg/day	300 ng/kg/day	
ENDOCRINE SYSTEM							
PITUITARY							
Cyst, pars distalis.		(25)	(25)	(25)	(25)	(25)	(24)
minimal		5	2	3	1		1
slight			2				
Score Expanded Totals		5	4	3	1		1
Within normal limits		20	21	22	24	25	23
THYROID							
Inflammation, artery.		(25)	(25)	(25)	(25)	(25)	(25)
moderate							1
Score Expanded Totals							1
Infiltrate, lymphocyte.							1
subimal		2				1	1
Score Expanded Totals		2				1	1
Ectopic thymus.							1
minimal				1	1		
slight		1				1	
Score Expanded Totals		1		1	1	1	
Cyst, follicular.							1
minimal		1					1
slight					1		
Score Expanded Totals		1			1		1
Within normal limits		21	25	23	23	23	22
GENITOURINARY SYSTEM							
CERVIX							
Within normal limits					(25) 25	(25) 25	(25) 25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICG007:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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 PLACES 2003 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
EPIDIDYMIDES							
Vacuolation, epithelium, increased, focal.							
minimal							
Score Expanded Totals	(25)	(25)	(25)				
Oligospermia							
slight							
moderate			1				
marked						1	
Score Expanded Totals	1	1	1			1	
Lymphangiectasis							
minimal			5			1	
Score Expanded Totals	2	5	5			1	
Inflammation, interstitium, chronic							
minimal							
Score Expanded Totals	1						
Infiltrate, lymphocyte							
minimal			12			7	
slight			1			3	
Score Expanded Totals	7	12	13			10	
Hyperplasia, epithelium, focal							
minimal							
Score Expanded Totals	2						
Granuloma, spermatic							
slight			1				
moderate			1				
marked						1	
Score Expanded Totals	2	2	2			1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p51 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males				Females			
		Feed w/ Iron		No Supplement		Feed w/ Iron		No Supplement	
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM									
EPIDIDYMIDES									
Fibrosis, interstitium.		(25)	(25)	(25)					
minimal		1		1					
slight		2	1	1					
Score Expanded Totals		3	1	2					
Edema, interstitium.									
minimal				1					
slight			1	1					
Score Expanded Totals			1	1					
Degeneration, epithelium, focal.									
minimal				1					
Score Expanded Totals				1					
Debris, increased, lumen.									
minimal		1	1						
slight		1							
Score Expanded Totals		2	1						
Cleft, cholesterol.									
slight			1						
Score Expanded Totals			1						
Within normal limits		11	9	13					
KIDNEY									
Increase, mesangial cell.		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
minimal		1		2					
Score Expanded Totals		1		2					
Vacuolation, tubular, epithelium, diffuse.									
marked				1					
Score Expanded Totals				1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICI687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, tubular, epithelium, focal.							
minimal		14	16			1	1
slight		6	4				1
Score Expanded Totals		20	22			1	2
Eyknoosis, mesangial cell.							
minimal			1	1			
Score Expanded Totals			1	1			
Mineralization, focal.							
minimal		7	8	2	3	1	1
Score Expanded Totals		7	8	2	3	1	1
Infiltrate, lymphocyte.							
minimal		6	12	13	10	7	7
slight			3	1	4	5	3
Score Expanded Totals		6	15	14	14	12	10
Hyalinization, glomerulus.							
minimal		1	3	3	2		
Score Expanded Totals		1	3	3	2		
Hyaline droplets, tubular, epithelium.							
moderate					1		
Score Expanded Totals					1		
Fibrosis, focal.							
minimal					1		
Score Expanded Totals					1		
Ectopic tissue, bone.							
minimal					2		1
Score Expanded Totals					2		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Deposition, fat, glomerulus.							
minimal		1	4	1			
Score Expanded Totals		1	4	1			
Cast, proteinaceous.							
minimal		8	5	8	10	11	6
Score Expanded Totals		8	5	8	10	11	6
Eosinophilic tubules.							
minimal		7	11	9	5	5	8
slight					1		
Score Expanded Totals		7	11	9	6	5	8
Within normal limits		1		5	6	5	6
OVARY					(25)	(25)	(25)
Hyperplasia, tubulointimal.							
minimal					2	1	1
slight						1	1
Score Expanded Totals					2	2	2
Hyperplasia, lymphoid.							
minimal					1	1	1
slight					1		
Score Expanded Totals					2	1	1
Hyperplasia, cystic, papillary.							
minimal						1	
Score Expanded Totals						1	
Hemorrhage, uterine.							
minimal					12	6	4
slight						2	
Score Expanded Totals					12	8	4

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICH607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
GENITOURINARY SYSTEM							
OVARY					(25)	(25)	(25)
Dilatation, oviduct.							
minimal						1	
Score Expanded Totals						1	
Cyst.							
minimal						1	1
slight					1	2	2
Score Expanded Totals					1	3	3
Within normal limits					13	11	16
PROSTATE		(24)	(25)	(25)			
Content, inspissated, focal.							
minimal		2	3	2			
slight		2	2	1			
moderate		1					
Score Expanded Totals		5	5	3			
Vacuolation, epithelium, focal.							
minimal			1				
Score Expanded Totals			1				
Lymphangiectasis.							
slight			1				
Score Expanded Totals			1				
Infiltrate, lymphocyte, ganglion.							
minimal		3	1				
slight			1				
moderate		1		1			
marked		1					
Score Expanded Totals		5	2	1			
Infiltrate, lymphocyte.							
minimal		8	12	10			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 ng/kg/day	0 ng/kg/day	200 ng/kg/day	0 ng/kg/day	0 ng/kg/day	300 ng/kg/day
GENITOURINARY SYSTEM							
PROSTATE							
Infiltrate, lymphocyte, slight		(24)	(25)	(25)			
Score Expanded Totals		5	3	5			
Fibrosis, interstitial, minimal		13	15	15			
Score Expanded Totals				1			
Ectasia, minimal		1	1	1			
Score Expanded Totals				1			
Ectasia, slight		1	1	2			
Score Expanded Totals		1	1	10			
Within normal limits		8	9				
SEMINAL VESICLE							
Dysplasia, epithelium, minimal		(25)	(25)	(25)			
Score Expanded Totals				1			
Metaplasia, coagulating gland, epithelium, focal, minimal			1				
Score Expanded Totals			1				
Infiltrate, lymphocyte, minimal		3	1	1			
Score Expanded Totals		3	1	1			
Dilatation, coagulating gland, minimal		3	2	1			
Score Expanded Totals		1	2	3			
Score Expanded Totals		4	10	4			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCY OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	NoSupplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	NoSupplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
GENITOURINARY SYSTEM							
ESPIDIAL VESICLE		(25)	(25)	(25)			
Dilatation.							
minimal				1			
slight				1			
Score Expanded Totals				2			
Within normal limits		18	13	18			
TESTIS		(25)	(25)	(25)			
Mineralization, focal.							
minimal		1					
Score Expanded Totals		1					
Dilatation, seminiferous tubule.							
minimal				3			
slight			1	1			
Score Expanded Totals			1	4			
Degeneration, seminiferous tubule.							
minimal		1	3	1			
slight		1					
moderate				1			
Score Expanded Totals		2	3	2			
Within normal limits		22	21	20			
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)
Thickened, submucosa.							
minimal				1			
Score Expanded Totals				1			
Inflammation, mixed cell.							
minimal							1
Score Expanded Totals							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICH607: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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		INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
ORGAN/TISSUE Finding	TREATMENT	Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)
Infiltrate, lymphocyte, submucosa.							
minimal		2	4	4	7	6	7
slight					7	2	
moderate						2	1
marked							1
Score Expanded Totals		2	4	4	14	10	9
Hyperplasia, transitional cell, diffuse.							
minimal			1	1			
Score Expanded Totals			1	1			
Within normal limits		23	20	20	11	15	15
UTERUS					(25)	(25)	(25)
Hyperplasia, cystic endometrial.							
minimal					10	16	8
slight					1	1	1
Score Expanded Totals					11	17	9
ENDOMETRIAL STROMAL POLYP (B).							
Atrophy, endometrium.					1	1	
slight							1
Score Expanded Totals							1
Within normal limits					14	8	15
VAGINA					(25)	(25)	(25)
Necification.							
minimal							1
slight					1		2

(B) Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 JCL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027H117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 300 ng/kg/day
GENITOURINARY SYSTEM							
VAGINA							
Mucification.					(25)	(25)	(25)
Score Expanded Totals					1		3
Hyperplasia, epithelium.							
minimal					3	2	1
Score Expanded Totals					3	2	1
Within normal limits					21	23	21
HEMOLYMPHATIC SYSTEM							
BONE MARROW							
Increase, megakaryocyte.		(25)	(25)	(25)	(25)	(25)	(25)
minimal						1	1
Score Expanded Totals						1	1
Increase, fat.							
minimal			1				
Score Expanded Totals			1				
Decrease, fat.							
minimal						2	1
Score Expanded Totals						2	1
Pigment, macrophage.							
minimal		10	1	4	10	2	1
slight		2			4		
Score Expanded Totals		12	1	4	14	2	1
Hyperplasia, lymphoid.							
minimal			1		2		1
Score Expanded Totals			1		2		1
NEUTROPHILIC SARCOMA (M).					1		

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
BONE MARROW		(25)	(25)	(25)	(25)	(25)	(25)
Granulopoiesis, increased.							
minimal				1	3	4	5
slight						1	
moderate							1
Score Expanded Totals				1	3	5	6
Fibrosis, focal.							
slight						1	
Score Expanded Totals						1	
Within normal limits		13	22	21	7	16	16
LYMPH NODE - AXILLARY LYMPHOMA [M].					(1) 1		
LYMPH NODE - BRONCHIAL		(23)	(25)	(25)	(25)	(23)	(25)
Plasmacytosis.							
minimal		3	1	3	2	1	1
moderate							1
Score Expanded Totals		3	1	3	2	1	2
Pigment, macrophage.							
minimal		6	13	6	12	12	9
slight		3	3	1	4	1	1
Score Expanded Totals		9	16	7	16	13	10
Necrosis, lymphoid.							
moderate				1			
Score Expanded Totals				1			
LYMPHOMA [M].					1		
Lymphangioectasis.							
minimal		3	7	5	1		1

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607: 26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - BRONCHIAL		(23)	(25)	(25)	(25)	(23)	(25)
Lymphangiectasis.							
slight		2	2	1			
Score Expanded Totals		5	9	6	1		1
Infiltrate, mast cell, increased.							
minimal		5	9	4	2	1	4
slight		2	2	4			
Score Expanded Totals		7	11	8	2	1	4
Hyperplasia.							
minimal		6	3	2	1		
slight		1					
Score Expanded Totals		7	3	2	1		
Histiocytosis.							
minimal			1	1	1	1	
Score Expanded Totals			1	1	1	1	
HISTIOCYTIC SARCOMA [N].							
Within normal limits		3	5	7	6	8	11
LYMPH NODE - MANDIBULAR		(23)	(22)	(22)	(25)	(24)	(25)
Extramedullary hematopoiesis.							
minimal				1			
Score Expanded Totals				1			
Pigment, macrophage.							
minimal					1	1	
Score Expanded Totals					1	1	
LYMPHOMA [N].							
Lymphangiectasis.							
minimal				1			
slight				1			

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICH607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MANDIBULAR		(23)	(22)	(21)	(25)	(24)	(25)
Lymphangiectasis.							
Score Expanded Totals				2			
Infiltrate, mast cell, increased.							
minimal		5	3	7	2	1	3
slight		2	3				
Score Expanded Totals		7	6	7	2	1	3
Hyperplasia.							
minimal		2	1	1	2	1	2
slight		1		1	1		
Score Expanded Totals		3	1	2	3	1	2
Histiocytosis.							
minimal		9	6	8	9	11	12
slight		1	2	2			
Score Expanded Totals		10	8	10	9	11	12
Within normal limits		8	10	7	10	10	9
LYMPH NODE - MEDIASTINAL LYMPHOMA [M].					(1)		
LYMPH NODE - MESENTERIC		(25)	(25)	(25)	(25)	(25)	(24)
Pigment, macrophage.							
minimal					1		
Score Expanded Totals					1		
LYMPHOMA [M].					1		
Hyperplasia.							
minimal		3		1			
Score Expanded Totals		3		1			

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MESENTERIC		(25)	(25)	(25)	(25)	(25)	(24)
Histiocytosis							
minimal		1	1	1			
slight				1			
Score Expanded Totals		1	1	2			
Within normal limits		24	24	22	23	25	24
LYMPH NODE - PANCREATIC					(1)		
Pigment, macrophage							
marked					1		
Score Expanded Totals					1		
Infiltrate, neutrophil							
slight					1		
Score Expanded Totals					1		
Hemorrhage							
moderate					1		
Score Expanded Totals					1		
LYMPH NODE - RENAL					(1)		
LYMPHOMA [M]					1		
LYMPH NODE - SUBLINGUAL					(1)		
LYMPHOMA [M]					1		
THYMUS		(24)	(25)	(25)	(25)	(25)	(25)
Necrosis, medulla							
minimal				1			
Score Expanded Totals				1			

[M] Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
THYMUS		(24)	(25)	(25)	(25)	(25)	(25)
Necrosis, cortex.							
marked				1			
Score Expanded Totals				1			
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Hyperplasia, epithelial.							
minimal				1			
Score Expanded Totals				1			
Hyperplasia, lymphoid.							
minimal		2	2	2			
slight		2	2	2			
Score Expanded Totals		4	4	4			
HISTIOCYTIC SARCOMA (N).							
Cyst.					1		
minimal			2	1	1		
slight		1			1		
Score Expanded Totals		1	2	1	2		
Atrophy.							
moderate							1
Score Expanded Totals							1
Within normal limits		19	21	20	23	25	24
SPLEEN		(24)	(25)	(25)	(25)	(25)	(25)
Extramedullary hematopoiesis, increased.							
minimal				1	6	2	6
slight					2		1

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL667:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
SPLEEN		(24)	(25)	(25)	(25)	(25)	(25)
Extramedullary hematopoiesis, increased, marked					1		
Score Expanded Totals				1	9	2	7
Pigment, melanocyte, minimal			2	3	1		
slight		1	1				
moderate		1			1		
Score Expanded Totals		1	3	3	1		
Pigment, macrophage, increased, minimal				1	7	5	
slight		2			10		8
Score Expanded Totals		2		1	17	5	
Necrosis, lymphoid, minimal				1			
Score Expanded Totals				1			
Hyperplasia, lymphoid, minimal			2	2	1	1	
Score Expanded Totals		2	2	2	1	1	
HISTIOCYTIC SARCOMA (N)					1		
Depletion, lymphoid, minimal				1			1
Score Expanded Totals				1			1
Deformity, minimal		1					
slight		1					
Score Expanded Totals		2					

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL607, 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males				Females	
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
SPLEEN		(24)	(25)	(25)	(25)	(25)	(25)
Congestion, moderate				1			
Score Expanded Totals				1			
Within normal limits		18	20	19	6	19	13
SYSTEMIC/ANY SITE		(25)	(25)	(25)	(25)	(25)	(25)
NEST CELL TUMOR (B)				1			
Lymphoma (M)					1		
HISTIOCYTIC SARCOMA (N)					1		
Within normal limits		25	25	24	23	25	25
INTERSTENTIAL SYSTEM							
CLYTICAL GLAND					(20)	(22)	(21)
Inflammation, mixed cell, minimal					6	5	2
Score Expanded Totals					6	5	2
Ectasia, minimal							1
Score Expanded Totals							1
Within normal limits					14	17	18
PANCREAS GLAND		(3)	(2)	(1)	(25)	(25)	(25)
Hypoplasia, duct, minimal							1
Score Expanded Totals							1
Within normal limits		3	2	1	25	24	25

(B) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 300 ng/kg/day
INTELLUMENTARY SYSTEM							
PROSTATIC GLAND		(25)	(25)	(25)			
Inflammation, mixed cell.							
minimal		2		1			
Score Expanded Totals		2		1			
Inflammation, chronic.							
minimal		3	1	9			
Score Expanded Totals		3	1	9			
Hyperplasia, epithelium, duct.							
minimal		1	1	1			
Score Expanded Totals		1	1	1			
Hyperplasia, acinar, focal.							
minimal		1	1				
Score Expanded Totals		1	1				
Ectasia.							
minimal		2	4	7			
slight			1	1			
Score Expanded Totals		2	5	8			
Atrophy.							
minimal		1	2	1			
slight				1			
Score Expanded Totals		1	2	2			
Within normal limits		16	19	10			
SKIN		(25)	(25)	(25)	(25)	(25)	(25)
Metaplasia, cartilage, pinna, focal.							
minimal				1	1	2	3
slight						1	
Score Expanded Totals				1	1	3	3

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
INTEGUMENTARY SYSTEM							
SKIN		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell, subcutis.							
minimal						1	
Score Expanded Totals						1	
Inflammation, subcutis, pinna, focal.							
minimal				2		2	3
slight				2		1	
moderate				1			
Score Expanded Totals				5		3	3
Infiltrate, lymphocyte, fat.							
minimal		2	1	1			
slight				1			
Score Expanded Totals		2	1	2			
Hyperplasia, cartilage, pinna, focal.							
minimal				2	2	1	1
slight						1	3
Score Expanded Totals				2	2	2	4
Hyperplasia, epidermis, pinna, focal.							
minimal				1			1
slight				1			
Score Expanded Totals				2		1	1
Granulation tissue, dermis, focal.							
slight				1			
moderate				1			
Score Expanded Totals				2			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

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ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
	0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day	
INTEGUMENTARY SYSTEM							
SKIN		(25)	(25)	(25)	(25)	(25)	(25)
Fibrosis, cartilage, pinna, focal.							
minimal		1	1	3	2	4	
Score Expanded Totals		1	1	3	2	4	
Fibrosis, dermis, pinna, focal.							
minimal		1	1		1		2
slight			1	2	1	2	2
moderate						1	1
Score Expanded Totals		1	2	2	2	3	5
Cyst, inclusion, epidermal.							
moderate				1			
Score Expanded Totals				1			
Atrophy, hair follicle.							
minimal				1	1	1	1
moderate		1					
Score Expanded Totals		1		1	1	1	1
Abscess, epidermal.							
minimal				2			1
moderate				1			
marked				1			
Score Expanded Totals				3			1
Within normal limits		21	22	17	20	17	20
MUSCULOSKELETAL SYSTEM							
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)
Proliferation, periosteum, focal.							
minimal			1				
Score Expanded Totals			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL687: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-MAR-04 13:35
 Page : 35
 PLACSS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
MUSCULOSKELETAL SYSTEM							
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)
Hypertrophy, synovium.							
moderate		1					
Score Expanded Totals		1					
Within normal limits		24	24	25	25	25	25
BONE - STERNUM		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)
Regeneration, myofiber, focal.							
minimal		1	1	10	1	1	3
Score Expanded Totals		1	1	10	1	1	3
Regeneration, myofiber, near insertion at patella.							
minimal		1					
Score Expanded Totals		1					
Necrosis, fibrinoid, artery.							
slight							1
Score Expanded Totals							1
Mineralization, myofiber, focal.							
minimal			1	5			
Score Expanded Totals			1	5			
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Inflammation, mixed cell, focal, near insertion at patella.							
minimal		1					
Score Expanded Totals		1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 17:35
 Page : 36
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
MUSCULOSKELETAL SYSTEM							
MUSCLE		(25)	(25)	(25)	(25)	(25)	
Inflammation, mixed cell, focal, minimal		1			1	1	
slight					1		
Score Expanded Totals		1			2	1	
Inflammation, focal, chronic, minimal			1	2			
Score Expanded Totals			1	2			
Fibrosis, focal, near insertion at patella, slight		1					
Score Expanded Totals		1					
Fibrosis, focal, minimal					1		
Score Expanded Totals					1		
Degeneration, myofiber, focal, minimal				4		2	
Score Expanded Totals				4		2	
Within normal limits		23	23	13	22	24	
NERVOUS SYSTEM							
BRAIN		(25)	(25)	(25)	(25)	(25)	
Vacuolation, nucleus oblongata, focal, minimal				1			
Score Expanded Totals				1			
Mineralization, thalamus, focal, minimal		5	2	2			
Score Expanded Totals		5	2	2			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICD607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
 Page : 37
 PLACSS 2000 V1.020

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
NERVOUS SYSTEM							
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)
Mineralization, meninges, focal.							
slight				1			
Score Expanded Totals				1			
Inflammation, artery, meninges.							
moderate							1
Score Expanded Totals							1
Dilatation, ventricle.							
minimal		1		2		1	
Score Expanded Totals		1		2		1	
Within normal limits		19	23	21	25	24	24
NERVE - SCIATIC		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25
SPINAL CORD		(25)	(25)	(25)	(25)	(25)	(25)
Malacia, focal.							
minimal							1
Score Expanded Totals							1
Gliosis, focal.							
minimal							1
Score Expanded Totals							1
Cyst, meninges.							
minimal				1			
Score Expanded Totals				1			
Within normal limits		25	25	24	25	25	24

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 IDENT: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
 Page : 38
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
RESPIRATORY SYSTEM							
LARYNX							
Inflammation, mixed cell, minimal						(1)	
Score Expanded Totals						1	
LUNG							
Swelling, epithelial cell, bronchiolar, moderate		(25)	(25)	(25)	(25)	(25)	
Score Expanded Totals			1				
Inflammation, granulocytic, focal, minimal					1		
Score Expanded Totals					1		
Inflammation, mixed cell, focal, minimal			1				
Score Expanded Totals			1				
Inflammation, pleura, mixed cell, focal, minimal					1		
Score Expanded Totals					1		
Inflammation, parivascular, minimal			1	1		1	
Score Expanded Totals			1	1		1	
Infiltrate, lymphocyte, pleura, focal, minimal		1	1	2	2	1	
Score Expanded Totals		1	1	2	2	2	
Inflammation, mixed cell, focal, minimal		1	1				
Score Expanded Totals		1	1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICH#07:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

S-MNR-04 13:35
 Page : 39
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
RESPIRATORY SYSTEM							
LUNG		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		3	2	3	2	4	3
slight				1		2	1
Score Expanded Totals		3	2	4	2	6	4
Hyperplasia, bronchioloalveolar, focal.							
minimal				2			
Score Expanded Totals				2			
Histiocytosis, alveolar, focal, with crystals.							
minimal				2			
Score Expanded Totals				2			
Histiocytosis, alveolar, focal.							
minimal				1			
slight			1	1			
Score Expanded Totals			1	2			
HISTIOCYTIC SARCOMA (M).					1		
Fibrosis, pleura, focal.							
minimal				1			
Score Expanded Totals				1			
Fibrin, pleura.							
moderate				1			
Score Expanded Totals				1			
Crystal, hemoglobin, bronchiole.							
minimal			1				
Score Expanded Totals			1				
Within normal limits		20	21	14	20	18	18

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0290117w
 5-NAR-04 13:35
 Page : 49
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
RESPIRATORY SYSTEM							
TRACHEA		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell, minimal			1	1			
Score Expanded Totals			1	1			
Inflammation, submucosa, chronic, minimal		1	1	1	1		1
Score Expanded Totals		1	1	1	1	1	1
Within normal limits		24	23	23	24	24	24
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Swelling, epithelial cell, cornea, focal, minimal				1			
Score Expanded Totals				1			
Ptyosis, lens, minimal		25	24	22	18	19	20
Score Expanded Totals		25	25	24	18	19	25
Degeneration, fiber, lens, minimal		17	7	13	5	6	7
Score Expanded Totals		4	5	7	3	2	9
Degeneration, fiber, lens, marked				1			1
Score Expanded Totals		21	12	21	8	8	18
Cataract, minimal							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1.3
 ICL607: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:15
 Page : 41
 PLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Cataract.							
slight							1
moderate							1
marked							1
Score Expanded Totals							4
Within normal limits				1	7	6	
HARDERIAN GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Pigment, increased.							
minimal				1			
Score Expanded Totals				1			
Infiltrate, lymphocyte.							
minimal			3	5	8	7	6
slight		5		1			1
Score Expanded Totals		6	3	6	8	7	7
Hyperplasia, focal.							
minimal		1					
Score Expanded Totals		1					
Dilatation, acini.							
minimal		5	2	2	1		
Score Expanded Totals		5	2	2	1		
Within normal limits		16	20	19	16	18	18
LACRIMAL GLAND		(25)	(25)	(25)	(24)	(24)	(24)
Metaplasia, focal.							
minimal			4	3		1	
slight						1	
Score Expanded Totals			4	3		1	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2.3
 ICL507:26-week oral [gavage] carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
 Page : 42
 PLACES 2000 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day
SPECIAL SENSE ORGANS							
LACRIMAL GLAND							
Infiltrate, lymphocyte.		(25)	(25)	(25)	(24)	(24)	(24)
minimal		15	16	11	13	11	12
slight		10	7	11	4	3	4
moderate							1
Score Expanded Totals		25	23	22	17	14	17
Cyst.							
minimal				1			
Score Expanded Totals				1			
Atrophy, acinar.							
minimal		9	14	8	5	2	4
slight		15	7	10	1	1	2
moderate		1	3	3			
marked							1
Score Expanded Totals		25	24	21	6	3	7
Within normal limits				1	6	9	5

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

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**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Tamal Chakraborti
9/8/2005 02:14:05 PM
PHARMACOLOGIST

Jasti Choudary
9/9/2005 09:00:50 AM
PHARMACOLOGIST
Review of carcinogenicity study portion of the NDA



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-882

SERIAL NUMBER: Original Submission 000: April 29, 2005
000 BP: July 8, 2005
000 C: July 12, 2005

DATE RECEIVED BY CENTER: Original Submission 000: 05/02/05
000 BP: July 11, 2005
000 C: July 12, 2005

PRODUCT: Exjade® (Deferasirox, ICL670) Tablets for Oral Suspension

INTENDED CLINICAL POPULATION: Adult and pediatric patients aged 2 years and above

SPONSOR: Novartis Pharmaceuticals Corporations

DOCUMENTS REVIEWED: OOO: April 29, 2005 (Pharm/Tox)

REVIEW DIVISION: Division of Gastrointestinal and Coagulation Drug Products (HFD-180)

PHARM/TOX REVIEWER: Tamal K. Chakraborti, Ph.D.

PHARM/TOX SUPERVISOR: Jasti B. Choudary, B.V.Sc., Ph.D.

DIVISION DIRECTOR: Brian H. Harvey, M.D., Ph.D.

PROJECT MANAGER: Alice Kacuba, RN, MSN, RAC

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations:

- A. Recommendation on Approvability: From a preclinical standpoint, this NDA may be approved.
- B. Recommendation for Nonclinical Studies: Sponsor's maximum limit of [] a genotoxic impurity) in the drug substance was about 12.5 times more than the maximum allowable amount of [] ppm based on toxicology study. The sponsor may be asked to reduce the level [] or conduct a 2-week oral toxicity study with [] in rats to qualify this impurity in the drug substance at the proposed maximum limit of [] ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).
- C. Recommendations on Labeling: The sponsor may be asked to modify the proposed label of Exjade as suggested in the text of this review.

II. Summary of Nonclinical Findings:

- A. Brief Overview of Nonclinical Findings: The systemic toxicity of ICL670 was adequately evaluated following oral and intravenous administration in mice, rats and marmosets monkeys. ICL670 was also tested orally in juvenile mice and rats to support its use in pediatric patients aged 2 years and above. The potential genotoxicity of ICL670 was examined in an adequate battery of genotoxicity tests. The carcinogenic potential of ICL670 has also been examined in p53 (+/-) transgenic mice and Wistar Hannover rats. In addition, ICL670 has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats. Adequate safety pharmacology studies were also conducted with ICL670.

Subacute/subchronic and chronic toxicity studies were conducted in mice (4 weeks, oral), rats (2-Week, i.v. and up to 26-week, oral) and marmoset monkeys (2-week, i.v. and up to 39 weeks oral). The target organs of toxicity appeared to vary across species. The major target organs of toxicity revealed by the repeated dose toxicity studies in mice, rats and marmoset monkeys were the kidney (in all species tested), eye (mice and rats), heart (mice and rats), gastrointestinal tract (mice and rats), and gall bladder and bile duct (marmoset). Kidney findings generally consisted of renal tubular degeneration, cytoplasmic vacuolation of cortical tubular epithelial cells and tubular necrosis across all species tested. Cataracts were observed in adult rats, juvenile rats and mice. Myocardial changes (multifocal myocytolysis in the ventricle in mice and myocardial degeneration predominantly in male rats) were observed in mice and rats. Gastrointestinal effects (gastric

erosions/ulcers, inflammation) were observed in mice and rats. In addition, inflammatory and degenerative changes in the gallbladder and bile ducts were seen in some marmosets at high doses. The no-observed-adverse-effect-levels (NOAELs) in mice, rats and marmoset monkeys were considered as 100, 30 and 40 mg/kg/day, respectively. The target organs identified in neonatal/juvenile animals were consistent with those observed in adults. However, juvenile mice and rats appeared to be more sensitive to the toxicity of ICL670 when compared to adults.

ICL670 was negative in the Ames test and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests. ICL670 was not tumorigenic in rats or p53 (+/-) transgenic mice.

ICL670 was found to have no adverse effect on fertility and reproductive performance of male and female rats. It was not teratogenic in rats and rabbits and did not appear to have adverse effects on postnatal development in rats.

In conclusion, the non-clinical studies conducted with ICL670 appeared to adequately support the administration of ICL670 at the intended therapeutic dosage and in accordance with the proposed product labeling.

- B. Pharmacologic Activity: ICL670 is an N-substituted bis-hydroxyphenyl-triazole, a representative of a new class of tridentate iron (Fe) chelator. Two molecules of ICL670 bind to one ferric atom. It has higher affinity ($pM = 22.5$) for iron than deferiprone ($pM = 19.5$) and lower than that of Desferal[®] ($pM = 26.6$). In bile duct cannulated rats, it was shown to be five-fold more potent than desferal (Iron binding efficiency or IBE = 3- 4% vs. 18% for ICL670) and nine-fold more potent than deferiprone (L1) (IBE = 2% vs. 18% for ICL670). The effective oral dose was determined to be 32 mg/kg in rats. In iron-overloaded rats, ICL670 was found to be twice as effective as desferal (58%) and 5-fold more effective than deferiprone (19%) in reducing non-heme liver iron concentration (LIC). About 40% of the ⁵⁹Fe-ICL670 complex was excreted in the feces within 48 hr compared to 0.5%, 3%, and 67% (urine) in control, deferiprone, and desferal, respectively, confirming the stability of the complex *in vivo*. In the iron-overloaded marmosets, at 22 mg/kg dose, the *in vivo* iron chelating efficiency of ICL670 was found to be 29% compared to 2% and 5% for deferiprone and desferal, respectively. ICL670 induced iron excretion predominantly by the fecal route and approximately 10% were excreted through the urine. ICL670 was found to be highly selective for iron and does not appear to induce the excretion of zinc and copper. Overall, *in vitro* and *in vivo* pharmacology studies with ICL670 appear to support its intended use as indicated in the proposed label.
- C. Nonclinical Safety Issues Relevant to Clinical Use: Based on non-clinical findings, patients should be closely monitored for renal function and periodic

ocular testing. In addition, patients should also be monitored closely for stomach and gall bladder changes while on treatment with ICL670.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-882

Review number: 000

Sequence number/date/type of submission: 000/April 29, 2005/Initial

Information to sponsor: Yes () No (X)

Sponsor: Novartis Pharmaceuticals Corporation

Manufacturer for drug substance:

1. Novartis Pharma AG, Basel, Switzerland
2. Novartis Pharma AG, Pratteln, Switzerland

Reviewer name: Tamal K. Chakraborti, Ph.D.

Division name: Gastrointestinal and Coagulation Drug Products

HFD #: 180

Review completion date: September 28, 2005

Drug:

Trade name: Exjade® Tablets for Oral Suspension 125, 250 and 500 mg

Generic name: Deferasirox

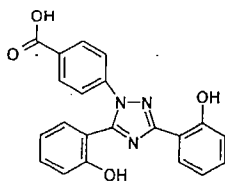
Code name: ICL670 (ICL670A, CGP72670, PN3002862)

Chemical name: 4-[3,5-Bis-(hydroxy-phenyl)-[1,2,4]-triazol-1-yl]benzoic acid

CAS registry number: 201530-41-8

Molecular formula/molecular weight: C₂₁H₁₅N₃O₄/373.37

Structure:



Relevant IND: IND 58,554 (ICL670, Novartis, HFD-180)

Drug class: Metal chelator

Intended clinical population: Adult and pediatric patients aged 2 years and above

Clinical formulation: ICL670 125, 250 or 500 mg Tablets for Oral Suspension. Each tablet contains standard excipients (lactose monohydrate, crospovidone, povidone, sodium lauryl sulfate, microcrystalline cellulose, silicon dioxide and magnesium

stearate). All excipients used were identified as either USP or NF. The composition is shown below (from page 64 of Chemistry Review dated July 6, 2005).

Table 2-1 Composition of one ICL670 dispersible tablet

Ingredient	Amount per tablet (mg)			Function	Reference to standards
	125mg tablet	250mg tablet	500mg tablet		
Deferasirox	125.0	250.0	500.0		
Croscopolidone					
Lactose monohydrate					
Lactose monohydrate					
Microcrystalline cellulose (NF)					
Povidone (K30)					
Sodium lauryl sulphate					
Colloidal silicon dioxide (NF)					
Magnesium stearate*					
Total tablet weight	425.0	850.0	1700.0		

Route of administration: Oral (Tablets for Oral Suspension)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: The following table shows the studies reviewed within this submission.

STUDY	REPORT/ STUDY NO.	TEST SITE	LOT NO.	REV. PAGE
PHARMACOLOGY*				11
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION				20
ABSORPTION				23
Absorption and disposition of [¹⁴ C]ICL670 in male rats	R97-522*	1	7408617, 7408618	23
Absolute bioavailability of three different oral formulations in dogs	R98-021	1, 3	CRP0520298 CRP0540298 CRP0550298 INJL 0921, 0926, 0927 and 0928	27
Absorption and disposition of [¹⁴ C]ICL670 in male marmosets	R98-315*	1	RSE 027	29

Absorption, distribution, metabolism and excretion of ICL670 in mice after single intravenous (10 mg/kg) and oral (10 mg/kg and 300 mg/kg) administration of [¹⁴ C]ICL670	R01-0012	1	RSE 027-11, E-0084-132, RSE 027-12, E-0084-133	30
Relative oral bioavailability of two formulations (CSF and MF) of ICL670 in dogs	R0101386	1	9923008	33
Comparative mechanistic transport studies with NVPAML644-AB-1 (ICL670 Fe-complex) and ICL670 across Caco-2 cell monolayers	R0201161	1	RSE 027-23, E-0084-194	33
Mechanistic transport studies across Caco-2 cell monolayers	R0200436	1	E-4885-077-24	34
Investigation of enterohepatic circulation in rats after intravenous administration of 10 mg/kg [¹⁴ C]ICL670	R0301123	1	RSE0270-23	35
Feasibility of the dog as food effect model of ICL670: effect of oil on oral bioavailability of parent drug and its iron chelate AML644	R0301126	1	VMLK/2002-0948	35
Determination of carrier-mediated cell uptake of ICL670 via hOATP1, hOATP2, hOATP8 and hOCT1 using pre-injected <i>Xenopus oocytes</i>	R0400372	1	E-12899-078-26	37
DISTRIBUTION				38
<i>In vitro</i> blood distribution and binding of ICL670 to plasma (or serum) proteins from human, dog, rat and marmoset	1998-039*	4	RSE 027	39
<i>In vitro</i> binding of ICL670 to albumin, α_1 -acid glycoprotein and gamma globulins (Phase I study)	R98-083*	4	RSE 027	39
Galactogenic transfer, kinetics and metabolism in milk and plasma after single oral administration of 10 mg/kg ¹⁴ C-labeled ICL670 to lactating rats	R00-0015	1	RSE 027	39
Placental transfer, kinetics and excretion of radioactivity in rabbits after oral administration of 50 mg/kg of [¹⁴ C] ICL670	R0101631	1	E-0084-108	40
Tissue distribution and embryofetal transfer in pregnant rats on Day 13 and Day 17 of gestation following a 30 mg/kg oral dose of [¹⁴ C]ICL670	R0101632	1	RSE 027-7	40
Distribution of radioactivity in sucking rat puppies and in the dams after single oral administration of 10 mg/kg [¹⁴ C]ICL670 to lactating rats	R0200702	1	RSE 027-13	41
Disposition of [¹⁴ C]AML644 (iron complex of ICL670) in male rats following a single intravenous dose of 10 mg/kg labeled compound	R0201158	1	RSE 192-2	42
Comparative analysis of <i>in vitro</i> blood distribution and plasma protein binding for [¹⁴ C]ICL670 and its iron complex [¹⁴ C]AML644 for rat, marmoset, rabbit, mouse, dog and human	R0201753	1	RSE 027-19	43
Disposition of [¹⁴ C]ICL670 in Wistar rats and in Mrp2-deficient (TR-) rats following a single intravenous dose of 10 mg/kg	R0300259	1	RSE 027-23	45
METABOLISM				47
<i>In vitro</i> biotransformation of ICL670 by liver microsomes of rat, rabbit, dog, marmoset, and man	1997-517*	1	RSE 027-1	47
Characterization of major metabolites of ICL670 formed in rats and marmosets after single oral and intravenous administration	R99-1771		RSE 027	48

<i>In vitro</i> biotransformation of [¹⁴ C]ICL670 in hepatocytes from rat and human	R0201160	1	E-15131-142-26	50
Determination of UDP glucuronosyltransferase (UGT) isoenzymes involved in the metabolism of ICL670	R0201162	1	E-0084-161	52
Analysis of the interconversion between ICL670 and its Fe ³⁺ complex, AML644, in rat blood and plasma	R0201163	4	NA	52
Assignment of metabolites of ICL670 in rat, mouse, marmoset and human ADME studies	R0400128	1	NA	53
Comparative systemic exposure to ICL670, iron-complexed ICL670 and 13 metabolites of ICL670 across species including man	R0400311	1	NA	54
Preliminary evaluation of an <i>in vitro</i> reporter gene assay for measurement of PXR-mediated induction of drug metabolizing enzymes	R0400619	1	0214012	61
EXCRETION				62
Excretion of iron with bile and urine after single intravenous administration of 50 mg/kg ICL670 and two of its metabolites (NVP-AHN496-NX-1 and NVP-AHK175-NX-1), respectively, to rats	R0200705	1	9923008	62
PHARMACOKINETIC DRUG INTERACTIONS				63
Inhibition of human P450 enzyme activities by ICL670 and its metabolite CGP82813A	DMPK(US) R99-077	5	NA	63
Identification of human P450 enzymes metabolizing ICL670A and determination of potential metabolic interactions on its metabolism	DMPK(US) R99-078	5	RSE 027-1	64
<i>In vitro</i> analysis of displacement of [¹⁴ C]warfarin or [¹⁴ C]diazepam from human plasma proteins by ICL670 and its iron complex AML644	R0400076	1	NA	66
Evaluation of ICL670 as an inhibitor of acetaminophen glucuronidation in human liver microsomes	R0400198	1	0214012	66
Interaction of ICL670 and its possible co-medication Hydroxyurea with MDR1, MRP2 and MXR efflux transporters	R0400371	1	NA	67
Evaluation of hydroxyurea as an inhibitor of ICL670 <i>in vitro</i> metabolism in human liver microsomes	R0400616	1	RSE 027-23	68
TOXICOLOGY				70
Acute				74
Mice				75
Oral	987027*	5	97902	75
Intravenous	987026*	5	97902	75
Rat				75
Oral	987010*	5	97902	75
Intravenous	987009*	5	97902	75
Subacute/Subchronic/Chronic				77
Mice				78
4-Week, oral	956185*	6	2	78
4-Week, oral	981060*	1	97902	80
4-week, oral	0170121*	5	9923008	83
Oral, juvenile	0480148	13	0213013	95
Rat				108
4-Week, oral	956214	6	NA	108
4-Week, oral	971055*	1	800196	109
4-Week, oral	987066*	5	97902	113

4-week, oral	974080*	6	800196	115
26-Week, oral	987037*	5	97902	123
2-Week, intravenous	0310064*	1	4469	129
Oral, juvenile, dose-ranging	0370003			136
Oral, Juvenile	0370030	5	9923008	138
Monkey				150
2-Week, oral	971974*	8	800196	150
2-Week, oral	971053*	1	800196	153
4-Week, oral, 4-week recovery	974194*	8	800196	155
4-Week, oral, palatability	982041*	8	97902	157
2-Week, intravenous	0320068*	10	4469	159
39-Week, oral	982027	9	98903	164
GENETIC TOXICOLOGY				171
<i>In Vitro</i>				171
Ames assay	CGP72670			171
Ames assay	956192	6	3	172
Ames assay	981683*	1	97902	173
Micronucleus test with V79 Chinese hamster cells	971709	11	1	173
Micronucleus test with V79 Chinese hamster cells	MIC127MNTV79	11	800196	175
Chromosomal aberration test in cultured human peripheral blood lymphocytes	981803*	1	97902	178
<i>In Vivo</i>				179
Micronucleus test, rat, oral	981866*	1	97902	179
Micronucleus test, rat, oral	981876*	1	97902	180
Micronucleus test, iron-overloaded rats, oral	981878*	1	98903	181
Induction of micronuclei in rat liver hepatocytes	982047*	12	97902	183
CARCINOGENICITY				185
104-Week, oral, rat	017022	5	9923008	185
26-Week, oral, p53 (+/-) transgenic mice	0270117	5	9923008	203
REPRODUCTIVE TOXICITY				229
Rat				230
Segment I, oral	987102*	5	97902	230
Segment II, oral	987055*	5	97902	232
Segment III, oral	997055	5	98903	240
Rabbit				237
Segment II, oral	987056	5	97902	237
LOCAL TOLERANCE				246
Intravenous, rabbit	0320046*	14	4469	246
SPECIAL TOXICOLOGY STUDIES				247
Ames test []	0113129	1	9040793	247
4-Week immunotoxicity, rat	0310048	1	9923008	248
Mechanistic study with kidney proximal tubular cell	971935	1	NA	251
Ames test with []	0212003	1	CHAD0101	253
Chromosome aberration test with cultured human peripheral blood lymphocytes with []	0212201	1	NA	256
<i>In vitro</i> cataract study with isolated rat lens	0480167	15	0321001	259
Toxicological qualification []				263

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*: Studies reviewed either under original submission of IND 58,554 dated June 30, 1999 or Amendments of IND 58,554. The pharmacology reviews of these studies are incorporated in the appropriate sections of this review.

Studies not reviewed within this submission: Analytical methods and validation reports were not reviewed, as these are reports of method development and validation.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

ICL670 is an N-substituted bis-hydroxyphenyl-triazole, a representative of a new class of tridentate iron (Fe) chelator. Two molecules of ICL670 bind to one ferric atom. It has higher affinity ($pM = 22.5$) for iron than deferiprone (L1) ($pM = 19.5$) and lower than that of Desferal[®] ($pM = 26.6$). In bile duct cannulated rats, ICL670 was shown to be five-fold more potent than Desferal (IBE = 3-4% vs. 18% for ICL670) and nine-fold more potent than deferiprone (IBE = 2% vs. 18% for ICL670). The effective oral dose was determined to be 32 mg/kg in rats. In iron-overloaded rats, ICL670 was found to be twice as effective as desferal (53%) and 5-fold more effective than deferiprone (19%) in reducing non-heme liver iron concentration. About 40% of the ⁵⁹Fe-ICL670 complex was excreted in the feces within 48 hr compared to 0.5%, 3%, and 67% (urine) in control, deferiprone, and desferal, respectively, indicating the stability of the complex *in vivo*. In the iron-overloaded marmosets, at equivalent (molar) doses (112 mg/kg, p.o.) the *in vivo* iron chelating efficiency of ICL670 was found to be 29% compared to 2% and 5% for deferiprone and desferal, respectively. ICL670 induced iron excretion predominantly by the fecal route and approximately 10% were excreted through the urine. ICL670 was

found to be highly selective for iron and does not appear to induce the excretion of zinc and copper.

In safety pharmacology studies, ICL670 caused deterioration in the step-through passive avoidance test (mice) and ataxia and head tremor in mice at 1000 mg/kg. ICL670 showed some affinities for kainate and the channel site of the NMDA receptor complex. In isolated guinea pig atria, ICL670 produced a marked decrease in heart rate at 300 μM concentration and increased the force of contraction at 100 μM concentration. In isolated sheep cardiac Purkinje fibers, ICL670 reduced maximum rate of depolarization, indicating an effect on cardiac sodium channels. In addition, ICL670 caused significant prolongation of APD₆₀ in isolated rabbit heart. However, ICL670 did not inhibit hERG current at 250 μM concentration. Overall, ICL670 appeared to have the potential to cause cardiovascular effects.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Characterization of ICL 670

ICL 670 is a tridentate ligand for Fe^{3+} (two molecules of ICL 670 binds to one ferric atom). It has higher (3 log units) affinity ($\text{pM} = 22.5$: pM : negative log [free Fe^{3+}], calculated for pH 7.4 at 1 μM Fe^{3+} and 10 μM ligand) for iron than LI (Kelfer®; CGP 37391), another orally active iron chelator, ($\text{pM} = 19.5$) but 4 log units lower than that of Desferal ($\text{pM} = 26.6$). In other words, ICL 670 binds iron about 1000 fold more tightly than LI.

Drug activity related to proposed indication:

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Induction of Iron Excretion in Non-Iron-Overloaded Rats

Induction of iron excretion was studied by oral gavage in bile duct cannulated male Tif:RAI rats treated with 25, 50, and 100 mg/kg of ICL 670. ICL 670 caused approximately 12-25-fold higher iron excretion compared to control within the first 3 hours of administration. About 85% or more of the total iron excreted was found in the bile and small amount was found in the urine. There was a dose-dependent increase in the excretion of iron in the bile and lasted for more than 24 hours at the highest dose. ICL 670 was found to be the most potent iron chelator. Its efficiency (the amount of excreted iron as percentage of theoretical iron binding capacity (IBE) of the dose) was determined to be 1875% (SD) at 50 and 100 mg/kg p.o compared to 3-4% and 2% for Desferal and L1, respectively. The "effective oral dose" (the dose which induces the excretion of 500 µg of iron/kg body weight in 24 hours) of ICL 670 was calculated to be about 32 mg/kg in Tif:RAI rats. ICL 670 was found to be less effective in Fisher rats than in Tif:RAI rats (used for toxicity studies).

12-Week Treatment of Iron-Overloaded Rats with the Iron Chelator ICL 670

The ability of ICL 670 to reduce body iron burden was investigated in iron-overloaded (0.1% ferrocene through diet for 5 weeks before drug administration) rats. Rats were treated with 59.7 (75 µmol/kg) and 270 mg/kg (330 µmol/kg) of ICL 670 orally for 12 weeks. Desferal and L1 (CGP37391) were administered at 98.4 (150 µmol/kg, s.c.) and 62.5 (150 µmol/kg, p.o.) mg/kg dose levels. ICL 670 reduced non-heme liver iron concentration by 53% (from 1256783 µg/g to 596772 µg/g) whereas Desferal and L1 caused 58% (1256783 to 5237110 µg/g) and 19% (from 1256783 to 1010746) reduction of iron concentration at twice the dose of ICL 670. The ferrocene-induced iron overload was completely reversed by ICL 670 at 75 µmol/kg and 150 µmol/kg by Desferal in 4 weeks. However, the higher dose of ICL 670 caused rapid depletion of kidney iron to below control level.

Fate of Iron Complex of ICL 670 in Rats

To examine the undesired redistribution of iron due to the breakdown of iron chelator complex prior to excretion, ⁵⁹Fe-ICL 670 complex was injected intravenously (i.v.) into rats. About 40% of the ⁵⁹Fe-ICL 670 complex were excreted in the feces within 48 hours compared to 0.5%, 3% and 67% (urine) in control (no chelator), L1, and Desferal, respectively. These results confirmed the stability of the complex *in vivo*.

Induction of Iron Excretion in Iron-Loaded Marmoset Monkeys

Marmosets (*Callithrix jacchus*) were iron-overloaded (8 weeks prior to the first treatment) by three intraperitoneal (i.p.) injections of iron dextran (separated by a 14-day interval after each injection, 200 mg/kg twice, 100 mg/kg the third injection). Marmosets were treated with 14 (18.8 µmol IBE/kg), 28 (37.5 µmol IBE/kg), 56 (75 µmol IBE/kg) and 112 (150 µmol IBE/kg) mg/kg orally. The control animals received 40% PEG 400 + 0.45% HPMC (hydroxypropyl-methylcellulose). A dose-dependent excretion of iron was primarily observed in the feces and considerable amount of iron was found in 24-48 hour period. From dose response studies, effective oral dose (ED) was found to be 22 mg/kg. The *in vivo* efficiency of iron chelation for

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ICL 670 was found to be 29% compared to 2% (marmosets and man) and 5-10% (man) for L1 and Desferal, respectively. The total iron excretion induced by oral ICL 670 was more than 10-fold higher than that of L1 at equivalent doses. ICL 670 at 150 μmol IBE/kg did not induce any excretion of copper and zinc in spite of having moderate affinities for copper and zinc.

ICL 670 is an N-substituted bis-hydroxyphenyl-triazole, a representative of a new class of tridentate iron chelator. Two molecules of ICL 670 bind to one ferric atom. It has higher affinity ($pM = 22.5$) for iron than L1 ($pM = 19.5$) and lower than that of Desferal ($pM = 26.6$). In bile duct cannulated rats, it was shown to be five-fold more potent than Desferal (IBE = 3-4% vs. 18% for ICL 670) and nine-fold more potent than L1 (IBE = 2% vs. 18% for ICL 670). The effective oral dose was determined to be 32 mg/kg in Tif:RAI rats. In a 12-week study in iron-overloaded rats, ICL 670 reduced non-heme liver iron concentration by 53% when compared to Desferal (58%) and L1 (19%) at twice the dose of ICL 670. In this study, ICL 670 was found to be twice as effective as Desferal and 5-fold more effective than L1. About 40% of the ^{59}Fe -ICL 670 complex was excreted in feces within 48 hr compared to 0.5%, 3%, and 67% (urine) in control, L1, and Desferal, respectively confirming the stability of the complex *in vivo*. In the iron-overloaded marmosets, at equivalent (molar) doses, the *in vivo* iron chelating efficiency of ICL 670 was found to be 29% compared to 2% and 5% for L1 and Desferal, respectively. ICL 670 induced iron excretion predominantly by the fecal route and approximately 10% were excreted through the urine. ICL 670 was found to be highly selective for iron and does not induce the excretion of zinc and copper.

2.6.2.3 Secondary pharmacodynamics

The sponsor did not provide any reports for secondary pharmacodynamic studies.

2.6.2.4 Safety pharmacology

Neurological effects:

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Safety Pharmacology

Evaluation of CNS Effects

ICL 670 was examined in mice and rats to detect possible CNS adverse effects. Single oral doses of 30, 100, 300 and 1000 mg/kg doses were administered in mice. No behavioral changes were observed compared to control at 30 mg/kg. However, a transient to weak ataxia (0.5 to 6h following 100, 300, and 1000 mg/kg) and marginal head tremor at 300 and 1000 mg/kg was observed at 1-2 hr post dose. No convulsions were observed in any group. Apomorphine-induced hypothermia (0.5 or 10 mg/kg, s.c.) or apomorphine (dopaminergic agonist, D1 and D2 agonist) induced climbing was not significantly antagonized by any of the test doses in mice indicating no potential anti-dopaminergic activity. Ethanol-induced narcosis (and loss of righting reflex) was not affected by any test dose in mice, which indicates that the drug does not appear to have potential hypnotic/sedative or anti-hypnotic activity. Step-through passive avoidance in mice was significantly deteriorated at 100 and 1000 mg/kg, but not at 30 or 300 mg/kg which indicate that the drug may have direct effects on memory and/or indirect effect through modulation of perception, motivation, emotion, etc.

In rats, the drug does not have any effect on rotarod test (indicative of interference with motor coordination, muscle relaxation and/or perception) at any tested dose level (30, 100, 300 or 1000 mg/kg orally). No significant drug induced changes in body-temperature were observed in rats. ICL 670 did not significantly affect horizontal (locomotor activity) or vertical activity (rearing) at any of the tested doses.

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Overall, ICL 670 following a single, oral administration caused some weak CNS effects in mice (transient ataxia and mild head tremor, deterioration of passive avoidance performance). It did not cause other CNS-related effects in rotarod test, body-temperature or motility in rats or ethanol-induced climbing, apomorphine-induced hypothermia or climbing in mice.

Interactions of ICL 670 at Neurotransmitter Receptors *In Vitro*

ICL 670 was examined for its interaction with different neurotransmitter receptors in *in vitro* receptor binding assays. ICL 670 did not interact (at 10 μ M) with α 1-, α 2- or beta adrenergic receptors, with 5-HT₁, 5-HT₂, and 5-HT₃ serotonergic receptors, with histamine-1, muscarinic, μ -type opiate receptor, benzodiazepine, adenosine-1, GABA-A, GABA-B, NK-1 or glycine binding site of the NMDA receptors. However, ICL 670 caused about 13% and 11% reduction in [³H]-kainate and [³H]-MK-801 (specific for channel site of NMDA receptor) binding at 10 μ M concentration, respectively.

Cardiovascular, Respiratory and Renal effects:

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Evaluation of Cardiovascular, Respiratory and Renal Effects of ICL 670

Possible pharmacological effects of ICL 670 on the arterial blood pressure, heart rate, electrocardiogram and on respiratory parameters (breathing rate, tidal and minute volumes) were studied in anaesthetized male rats over a period of 2.5 hours at 100, 300, and 1000 mg/kg (intraduodenal administration). ICL 670 was found to have no effect on any of those parameters at the tested doses. ICL 670 had no effect on renal excretion of chloride, sodium and potassium ion or urine excretion over a period of 6 hours up to 1000 mg/kg dose level in conscious female rats. Isolated guinea-pig atria were used to examine the *in vitro* effects of ICL 670 on heart rate and force of contraction at 30, 100, and 300 μ M concentrations. At 300 μ M concentration, the drug decreased heart rate by 56.2% and 69.7% below baseline values at 20 min and 30 min after drug administration, respectively suggesting a direct effect on the atrial tissue. Treatment-related changes in the force of contraction were observed at 100 and 300 μ M concentrations. The force of contraction was increased by 17.3% and 30.4% at 100 and 300 μ M concentrations at 10 min after drug addition, respectively followed by a progressive decrease below baseline levels at high dose (-21% at 30 min). These changes in the heart rate and force of contraction in *in vitro* experiments suggest potential adverse effect of ICL 670 on the cardiac tissue at 300 μ M concentration.

Effect of ICL 670 on Action Potential Parameters in Sheep Isolated Cardiac Purkinje Fibers

In standard physiological salt solution, ICL 670 at 8, 24, and 72 μ g/ml concentrations significantly decreased action potential duration (25% and 50% reduction at 24 and 72 μ g/ml, respectively), upstroke amplitude (10 and 20 mV difference at medium and high concentrations, respectively compared to control) and maximum rate of depolarization (10%, 21% and 36% reduction at 8, 24, and 72 μ g/ml, respectively) in sheep isolated Purkinje fiber preparation. However, in the presence of human plasma proteins, ICL 670 at 8, 24 and 72 μ g/ml did not affect action potential duration beyond the shortening observed with the vehicle alone. ICL 670 reduced the maximum rate of depolarization as mentioned above indicating an effect on cardiac sodium channels. This may reduce myocardial excitability at higher plasma concentration and cause a widening of the QRS complex, which may lead to conduction abnormalities in humans.

Electrophysiological Investigations in the Isolated Rabbit Heart

In this study, isolated rabbit heart was perfused according to the Langendorff technique. The His bundle was cut and two types of electrodes were implemented in the left ventricle (subendocardium and pericardium) to record monophasic action potentials (MAPs). The heart was superfused with increasing concentrations of the test article, at different stimulation rates. The recording of MAPs was conducted at various stimulation rates under control conditions and during test item superfusion to determine the effects on action potential duration (APD) and reverse rate-dependency of AP duration. ICL670 was tested at 0.3, 0.9, 3, 9 and 30 μ M in three separate experiments (13815, 13828 and 13813). The following parameters were measured: action potential duration at 30% (APD₃₀), 60% (APD₆₀) and 90% (APD₉₀) of repolarization, reverse use-dependency, instability, triangulation, proarrhythmia index coronary perfusion rate, intraventricular conduction, variability in pacemaker activity and amplitude of the threshold stimulation current.

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In experiment 13815, ICL670 induced a significant APD₆₀ prolongation (15.9% increase compared to control) at the 0.9 μ M. concentration. The prolongations of APD₆₀ in reference to control were: 8.2% (increase of 18 ms), 15.9% (increase of 35 ms), 9.9% (increase of 22 ms), 5.5% (increase of 12 ms) and 4.7% (increase of 10 ms) at 0.3, 0.9, 3, 9 and 30 μ M, respectively. The following table (page 16 of the report of the study 13815) shows the results of this study (13815).

13815	ICL670	Instab	Triang	RevUse	ProArr	INDEX	APD60
	0 μ M	5	105	-3	2	0	223
	.3 μ M	1	-12	-1	-2	-13	18
	.9 μ M	0	-19	-2	-1	-21	35
	3 μ M	-1	-28	-3	0	-32	22
	9 μ M	2	-15	-1	4	-10	12
	30 μ M	0	-19	-2	0	-21	10
	0 μ M	4	-36	-4	1	-34	19

In experiment 13828, ICL670 also caused prolongation of APD₆₀. The prolongations of APD₆₀ in reference to control were: 5.5% (increase of 10 ms), 7.5% (increase of 13 ms), 4.1% (increase of 7 ms), 4.5% (increase of 8 ms) and 5.2% (increase of 9 ms) at 0.3, 0.9, 3, 9 and 30 μ M, respectively. The following table (page 7 of the report of the study 13828) shows the results of this study (13828).

13828	ICL670	Instab	Triang	RevUse	ProArr	INDEX	APD60
	0 μ M	3	91	-2	6	0	182
	.3 μ M	3	-28	0	11	-14	10
	.9 μ M	0	-29	14	-5	-19	13
	3 μ M	0	-30	-2	7	-25	7
	9 μ M	2	-33	0	3	-27	8
	30 μ M	0	-30	0	0	-30	9
	0 μ M	-2	-38	-3	-1	-43	7

In study 13813, ICL670 also caused prolongation of APD₆₀. The prolongations of APD₆₀ in reference to control were: 7.2% (increase of 15 ms), 5.3% (increase of 11 ms), 7.5% (increase of 16 ms), 9.8% (increase of 21 ms) and 10.0% (increase of 21ms) at 0.3, 0.9, 3, 9 and 30 μ M, respectively. The following table (page 3 of the report of the study 13813) shows the results of this study (13813).

13813	ICL670	Instab	Triang	RevUse	ProArr	INDEX	APD60
	0 μ M	14	162	-8	0	0	217
	.3 μ M	-12	-33	2	1	-42	15
	.9 μ M	-11	-51	0	5	-57	11
	3 μ M	-10	-57	4	2	-60	16
	9 μ M	-9	-44	2	4	-47	21
	30 μ M	-9	-56	1	6	-57	21
	0 μ M	-8	-84	3	4	-84	23

The sponsor contended that these effects resulted from the squaring of the action potential shape (increase in the plateau height). In addition, the coronary perfusion rate increased during drug perfusion and the pacemaker activity slightly decreased at 30 μ M in study 13815. In another assay (13828), the threshold stimulation current increased at 30 μ M concentration. No significant effects were observed in the third experiment when compared to respective control values.

Overall, ICL670 caused significant prolongation of APD₆₀ in isolated rabbit heart. The results indicated that ICL670 might have proarrhythmic potential. In addition, ICL670 increased the coronary perfusion rate and the threshold stimulation current and decreased the pacemaker activity at 30 μ M.

Electrophysiological Safety Measurements of hERG Currents in Stably Transfected HEK293 Cells

The objective of this study was to assess the effect of ICL670 on tail current recorded from HEK293 cells stably transfected with hERG cDNA using the patch clamp technique. The effects of ICL670 (250 μ M), were investigated using six cells per concentration. ICL670 could not be tested in concentrations higher than 250 μ M because of its limited solubility in dimethyl sulfoxide (DMSO). The effect of vehicle (0.1% DMSO) and the effect of positive control (E-4031, 100 nM, a hERG channel blocker) were also examined to confirm the sensitivity of the test system. Generally, cells were exposed to each concentration for approximately 10 min. The magnitude of the steady state tail current was compared to the value obtained under control conditions and the residual current (% of control) was calculated. The values were then corrected for the observed appropriate mean vehicle rundown (n = 5).

In the vehicle treated cells (n = 5) exposure to 0.1% DMSO produced a residual tail current of 92.8 ± 1.9 % of control (1.27 nA), which equated to a decrease in tail current of 7.2 %. When administered to two of the 0.1 % DMSO treated cells, E-4031 (100 nM) produced a residual hERG tail current of 3.3 ± 1.7 % of control (1.61 nA). This equated to a decrease in tail current of 96.7%. ICL670 (250 μ M) produced a residual vehicle corrected hERG tail current of 114.5 ± 8.1 % of control (1.71 nA, n = 6). ICL670 did not appear to inhibit hERG channels in stably expressed in HEK293 cells at

concentration of 250 μ M. This is to be mentioned here that the sponsor only tested one concentration of ICL670. The sponsor stated that concentration higher than 250 μ M could not be tested because of the limited solubility of ICL670 in DMSO.

Telemetry Study in Dogs Including Sighting Phase

The purpose of this telemetry study in dogs was to obtain cardiovascular safety pharmacology information for ICL670 after single-dose administration to Beagle dogs.

Sighting phase

Single doses of ICL670 were administered orally (capsule and gavage) to male dogs (n = 2/group) using gelatin capsules or by gavage (in 0.5% w/v of Klucel HF) at dosages of 10 to 1000 mg/kg. The following table shows the study design (from page 10 of the study report).

Table 1-1 Animal allocation and test item dosages

Group no.	Sex	No. of animals	Method of administration	Study day	Dosage (mg base/kg)	Concentration (mg/mL)	Dosage volume (ml/kg)
1	male	2	capsule	1, 8	10, 100	Not applicable	Not applicable
			oral gavage	18, 32	100, 1000	50, 150	2, 6.7
2	male	2	capsule	3, 10	30, 300	Not applicable	Not applicable
			oral gavage	25, 39	300, 600	150	2, 4

The following parameters were observed: mortality, clinical signs, body weight, food consumption, electrocardiography, hematology, clinical biochemistry, urinalysis and toxicokinetic investigations. Administration of ICL670 up to 1000 mg/kg revealed no adverse effects in clinical parameters. However, based on the severity of treatment-related emesis at 600 mg/kg, this dose was considered prohibitive for telemetric evaluations. Toxicokinetic results revealed high inter-animal variability of plasma concentrations when ICL670 was administered using gelatin capsules. Dosages of 0 (vehicle only), 100 and 300 mg/kg were selected for administration by oral gavage in the telemetry phase.

Telemetry phase

Single doses of ICL670 were administered orally (gavage) to male dogs (n = 4/dose) at 100 and 300 mg/kg (2 ml/kg). The control animals received the vehicle (0.5% w/v aqueous Klucel HF). The following parameters were examined: mortality, clinical signs, body weight, food consumption, telemetry (heart rate, blood pressure, and electrocardiography/ECG: PR, QT and RR intervals).

There were no deaths following administration of ICL670 at 100 or 300 mg/kg and no treatment-related clinical signs or effects on body weight and food consumption.

Telemetric investigations revealed no treatment-related changes in diastolic or arterial blood pressure, core body temperature or ECG morphology at these dosage levels.

Gastrointestinal effects: The sponsor did not provide any report.

Abuse liability: The sponsor did not provide any report.

Other:

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Effect of ICL 670 on the Absorption of Dietary Iron in Rats

Iron chelators often facilitate the uptake of dietary iron by forming complex with dietary iron during transit through the gastro-intestinal tract. The potential of ICL 670 to increase the absorption of dietary iron was examined in rats. In this study, rats were treated with 40 mg/kg/day of ICL 670 for two weeks preceded by the intragastric application of small amounts of ^{59}Fe . ICL 670 treated rats showed less ^{59}Fe retention compared to control (8.55 γ 1.5% vs. 13.2 γ 0.8% in control). Radio-iron distribution study in different organs (blood, liver, spleen, heart, kidneys, femur, carcass) in these animals revealed that iron retention in the liver was considerably less compared to control (29 γ 8.7 nCi vs. 60.4 γ 20 nCi in the control).

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2.6.2.5 Pharmacodynamic drug interactions

The sponsor did not provide any report of pharmacodynamic drug interactions.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not included.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

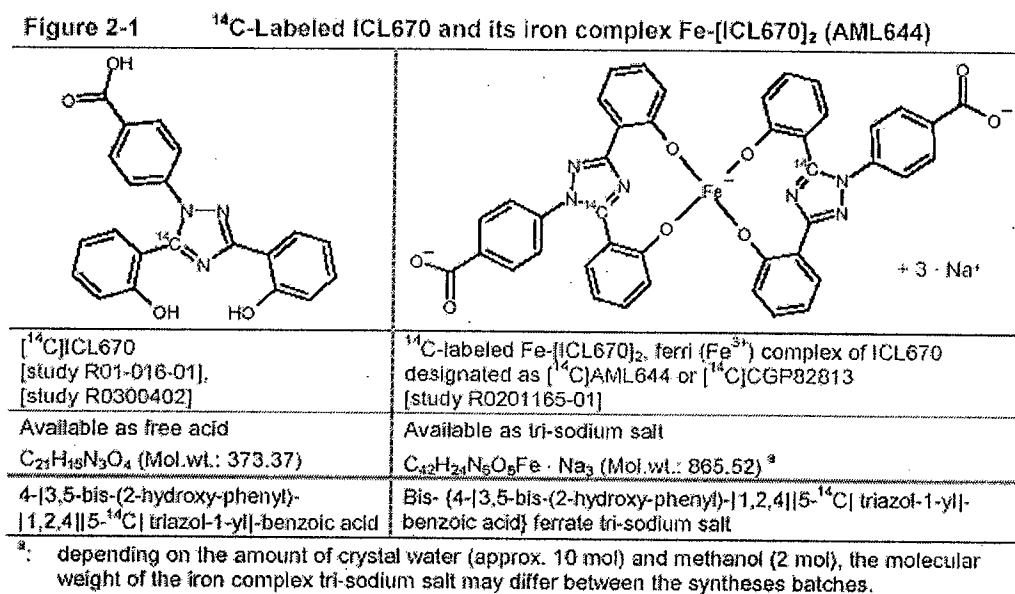
Absorption, distribution, metabolism and excretion (ADME) studies were conducted in mice, rats and marmosets. ICL670 was rapidly absorbed following single oral doses in mice, rats, dogs, marmosets and humans. The absolute oral bioavailability of total ICL670 (sum of ICL670 free and Fe-[ICL670]₂) ranged from 58% in mice, 32% in rats, 100% in dogs, 88% in marmosets and 73% in human. Enterohepatic recirculation of ICL670 was observed in bile duct-cannulated rats. ICL670 and its iron complex Fe-[ICL670]₂ were extensively bound to plasma proteins of all species tested including man. Across the species investigated, the plasma protein binding for ICL670 or iron complex Fe-[ICL670]₂ were greater than 98%. Binding to isolated human plasma proteins

indicated that serum albumin was the main binding protein (98%-99% bound). Generally, the iron complex Fe-[ICL670]₂ displayed a higher plasma protein binding than ICL670. In the whole blood, ICL670 was mainly confined to the plasma compartment. The fraction of ICL670 partitioned into blood cells was 5%-11% in human, rabbit and dog and 23%-37% in the mouse, marmoset and rat. No uptake into the blood cells was observed for the iron complex Fe-[ICL670]₂. The volume of distribution (V_{ss}) of ICL670 at steady-state was small to moderate in mice, rats, dogs and humans (0.18- 0.82 L/ kg). In whole body autoradiography tissue distribution studies in rats, up to 8 hours post-dose, most organs and tissues showed radioactivity (¹⁴C concentrations) close to or below than that observed in the blood. Highest levels of radioactivity were seen in the GI-tract and excretory organs (liver, kidney and intestine). Thereafter, radioactivity was eliminated from blood and most tissues with apparent half-lives ranging from 2 to 7 hours, except in the skin where a half- life of about 21 hours was seen. Measurable ¹⁴C concentrations were still found only in the liver and kidneys after 7 days with trace amounts in the skin. ICL670 and/or its metabolites crossed the blood-brain barrier to a relatively low extent. ICL670 crossed the placenta and excreted extensively into the milk in pregnant rats as unchanged ICL670. The ¹⁴C milk/plasma ratio at 4-8 hours was about 20, indicating substantial excretion of ICL670 in the breast milk. In addition, suckling juvenile rats (PND10) were also exposed to ICL670 and/or its metabolites between one and at least 24 hours after oral administration of 10 mg/kg of [¹⁴C] ICL670 to female lactating rats. Overall, it appears that ICL670 has the potential to be excreted through human milk. In liver microsomes from the rat, rabbit, marmoset, dog and human, oxidative biotransformation of ICL670 caused formation of mainly M1 (5-hydroxy metabolite of ICL670) and M2 (3-hydroxy), and to a minor extent M4 (5'-hydroxy metabolite) and M5 (3'-hydroxy metabolite). Formation of M1 and M2 *in vitro* was catalyzed by CYP1A1 and CYP1A2 and M4 and M5 were formed by CYP2D6. M1 and M2 were found to be about 6-fold weaker than ICL670 to complex and eliminate iron. In human and rat hepatocytes, oxidative and conjugative biotransformation of ICL670 was observed. The acyl-glucuronide conjugation of ICL670 (M3) was an important biotransformation pathway. Additionally, metabolites M7 (5-O-sulfate metabolite) and M4 (5'-hydroxy metabolite) were also observed. However, metabolite M6 (2-O-glucuronide metabolite) was seen in human hepatocytes only. UGT1A1 and UGT1A3 were the predominant isoenzymes involved in the glucuronidation of ICL670 accounting together for about 95% of the glucuronide formation. In *in vivo* metabolic studies in mice, rats, marmosets and humans, oxidative metabolism of ICL670 was important in the rat and marmoset and was found to be low in the human and glucuronidation was also predominant in rodents, marmosets and humans. Overall, in all tested species including human, biotransformation was extensive with formation of primary (Phase I) and conjugated (Phase II) metabolites, the major metabolic pathways being hydroxylation, glucuronidation and sulfation. *In vitro* studies with efflux inhibitors suggested that ICL670 was a substrate for multidrug resistance related protein 2 (MRP2) and BCRP (breast cancer resistant protein). Hence, ICL670 may have some weak MRP2 and BCRP-mediated drug-drug interaction potential. Multidrug resistance (MDR1) glycoprotein (P-glycoprotein) showed no transport activity. ICL670 weakly inhibited activities of some human cytochrome P450 isoenzymes e.g. CYP2C8, CYP1A2, CYP3A4/5, CYP2D6, CYP2A6, CYP2C9 and CYP2C19 with IC₅₀ values between 100

and 340 μM . Drug-drug interactions with ICL670 may occur by competition for UDP (uridine 5'-diphospho)-glucuronosyltransferase (UGT1A1 or UGT1A3). Biotransformation of ICL670 was not inhibited by hydroxyurea. Hence, an interaction of ICL670 and hydroxyurea in patients appeared to be unlikely. Irrespective of the route of administration, in all tested species, excretion of radioactivity after i.v. and p.o. dosing occurred predominantly via bile/feces and was virtually complete within 3 days (mice) or 7 days (rats, marmosets). In the rat, 58-76% of the dose was excreted in the bile. Renal excretion generally accounted for less than 12% of dose.

2.6.4.2 Methods of Analysis

ICL670 and its iron complex $\text{Fe}[\text{ICL670}]_2$ were ^{14}C -labeled in the 5-position of the triazole ring of the molecule. The following figure (from page 11 of the summary report) shows the position of radiolabeling.



The ^{14}C radioisotope in the molecule was shown to be chemically and metabolically stable. Concentrations of total ICL670 and its iron complex $\text{Fe}[\text{ICL670}]_2$ in the plasma, and ICL670 in urine and tissues of animal and human plasma were determined using validated and specific high pressure liquid chromatography (HPLC) methods with ultraviolet detection (HPLC-UV) or γ counting. The lower limit of quantitation (LLOQ) values for ICL670 and $\text{Fe}[\text{ICL670}]_2$ in the plasma were typically 100 ng/L and 10 ng/L , respectively.

ICL670 and its iron complex $\text{Fe}[\text{ICL670}]_2$ were found to be stable in the plasma of animals and man; however, the complex could not be determined in the rat bile or feces due to its inherent instability in these biological matrices. Hence, investigation and

interpretation of elimination processes were limited and were based on total ICL670 (sum of free and iron-complexed ICL670) and its metabolites.

Specific methods of analysis were also discussed under individual study reviews.

2.6.4.3 Absorption

Absorption

Rat

Absorption and Disposition of [¹⁴C]-ICL 670 in Male Rats

Methods: Male albino HAN:WIST (SPF) and LE (SPF) intact or bile duct cannulated rats received [¹⁴C]-ICL 670 by the intravenous (i.v.) route at 10 mg/kg (Batch no. 7408617, specific activity = 1.76 MBq/mg) and by oral (p.o.) route at 10 (Batch no. 7408618, specific activity = 0.364 MBq/mg) and 100 mg/kg (Batch no. 7408617A, specific activity = 0.176 MBq/mg. This batch was prepared by dilution of Batch 7408617 with non-labeled ICL 670, Lot No. 800196) dose levels. There were 3 rats/dose group. Urine and feces were collected at daily intervals up to 168 and 72 hours post dose from intact and bile duct cannulated rats, respectively. The absorption, tissue distribution, kinetics, biotransformation and excretion of ICL 670 were studied. Radioactivity in all samples was determined by liquid scintillation counter (LSC). Tissue distribution was studied by quantitative whole body autoradiography (QWBAL). Unchanged ICL 670 and its iron complex were determined by high pressure liquid chromatography (HPLC). Metabolite patterns were determined in plasma, bile, urine and feces by using HPLC with radioactivity detection.

Results: The pharmacokinetic parameters of [¹⁴C]-ICL 670, unchanged ICL 670 and its iron complex CGP82813 in plasma are shown below (reproduced from vol. 19, page 8-4874 of sponsor's submission):

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Dose (mg/kg) /Route	Tmax (hr)			Cmax (µmol/L)			Specific AUC ^b [µmol/L-h/(mg/kg)]		
	¹⁴ C	Unchan- ged ICL 670	Iron Comp lex CGP8 2813	¹⁴ C	Unchan- ged ICL 670	Iron Comp lex CGP8 2813	¹⁴ C	Uncha- nged ICL 670	Iron Compl ex CGP8 2813
10 (i.v.)	0.083 ^a	0.083 ^a	1	61.3	67.7	2.35	10.3	7.96	0.49
10 (p.o.)	0.5	0.5	2	8.51	7.11	0.57	4.02	2.04	0.67 ^c
100 (p.o.)	1-8	1	4	151	149	7.07	23.5	21.6	0.91 ^c

- a. First sample taken.
- b. Intravenous: 0.08-24h; Oral: 0-24h
- c. The part of AUC extrapolated between 8 h (last quantifiable data point) and 24 h (concentration below LOQ, taken as zero) was high. It represented about 50% of AUC (0-24h).

About 75% of the administered dose were absorbed after oral absorption based on the combined biliary and urinary excretion data. Based on the AUCs of ICL 670 in plasma after i.v. and p.o. administration, the bioavailability following 10 and 100 mg/kg p.o. in rats were determined to be 26% (2.04* 100/7.96 = 26%) and 27% (21.6* 100/10* 7.96 = 27.13%, dose normalized for 10 mg/kg), respectively, taking the i.v. 10 mg/kg dose as reference. The sponsor's reported value of 270% bioavailability based on 100 mg/kg oral dose should be corrected for normalization of dose for 10 mg/kg. Concentration of iron complex CGP82813 accounted for 3-29% of unchanged ICL 670.

Distribution of [¹⁴C]-ICL 670 in Organs and Tissues of Male Rats

The whole body autoradiographic studies revealed that the radioactivity was moderately distributed throughout the body. At 1 and 24 hr postdose the ¹⁴C concentrations in different organs and tissues were similar to the concentration in blood except liver (4-10-fold higher concentration than blood) and kidney (2-10-fold higher concentration than blood). The maximum concentration after 10 mg/kg oral dose was observed at 4 hr postdose in the kidney, liver and intestine (8-12 nmol/g). The distribution pattern after 8 hr was similar to that after 4 hr and decreased to background values within 24 hr postdose except kidney (cortex) and the skin where maximum radioactivity (1.5 nmol/g) was found at 48 hr postdose and was detected up to the end of the experimental period. After 100 mg/kg oral dose, the maximal concentrations of radioactivity was found in the liver (750 nmol/g) and kidney (cortex, 630 nmol/g) at 4 hr postdose. Most tissue concentrations were in the range of 60-300 nmol/g except for the brain, vitreous body and white fat (8 nmol/g). The pattern of disappearance of radioactivity from different organs was similar to that observed following 10 mg/kg dose. At 24-hr postdose of 100 mg/kg, skin (65 nmol/g), kidney (20 nmol/g) and liver (10 nmol/g) had considerable amount of radioactivity. Generally, the distribution pattern after the low (10 mg/kg) and high (100 mg/kg) oral doses were comparable. The amount of radioactivity in blood, liver, and kidney at 1 and 24 h following 10 and 100 mg/kg oral doses are shown in the following table:

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Sample	¹⁴ C Concentration (nmol/g)			
	10 mg/kg		100 mg/kg	
	1 hour	24 hour	1 hour	24 hour
Blood (µmol/L),	15	150	0.05	0.54
Liver	104	0.50	536	4.95
Kidney	55.8	0.54	304	3.76

The distribution of radioactivity (as determined by quantitative whole body autoradioluminography in one animal per time point) after po (10 and 100 mg/kg) in different organs at 4, 8, 24, 48, and 168 hours after drug administration is shown in the following table:

Sample	¹⁴ C Concentration (nmol/g)				
	4 h	8 h	24 h	48 h	168 h
10 mg/kg					
Liver	10.9	10.2	Nd	Nd	Nd
Renal cortex	12.6	8.21	*	Nd	Nd
Large intestine	9.04	Nd	Nd	Nd	Nd
Skin	*	0.54	0.97	1.54	*
100 mg/kg					
Liver	765	581	11		Nd
Renal cortex	631	432	23		Nd
Skin	182	128	65		*

Nd: Below detection limit.

*: Between quantitation limit (— nmol/g) and detection limits (— µmol/g).

Metabolism of [¹⁴C]-ICL 670 in Male Rats

ICL 670 was extensively metabolized in the liver. Several primary (Phase I) and conjugated (Phase II) metabolites were detected. About 80-95% of the parent compound was found in the plasma. Similar metabolite pattern in plasma was observed following oral and intravenous administration of ICL 670. Bile was analyzed from bile duct-cannulated rats. Two major metabolites (hydroxylated and acyl glucuronide of ICL 670) were detected in the bile. In the feces, only parent compound was detected. The feces of the non-bile duct-cannulated rats contained 90% of the dose (20-50% was unchanged drug and 5-11% of three different metabolites). The concentrations of major metabolites (peaks) in the plasma after 10 and 100 mg/kg, p.o. are shown in the following table:

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Sample	AUC 0-8h (µmol.h/L) of Major Peaks in Plasma				
	P55.6	P70.7	P86	P82 (Parent)	P63.3
10 mg/kg					
Plasma	1.6	0.9	1.4	21.3	
100 mg/kg					
Plasma	6.5	38.4	0	920.8	9.4

Pattern of some major radioactive metabolites in bile, feces, and urine collected after single oral doses of 10 mg/kg of radioactive ICL 670 as determined by HPLC-radiometry are shown in the following table:

Sample Type	Bile	Feces	Urine	Total Excretion
Excretion Period	0-48	0-48	0-48	0-48
Peak	Excretion (% of Dose)			
P46.2	7.4	0	0	7.4
P48.8	5.4	0	0.7	6.1
P49.7	3.7	0	0.3	4.0
P52.0	0.8	0	0.2	1.0
P54.3	2.8	0	0.7	3.5
P68.9	26.6	0.1	0.2	26.9
P70.7	2.8	0.1	1.6	4.6
P82 (Parent)	7.7	14.9	0.1	22.7

Excretion of Radioactivity in Urine, Bile and Feces in Rats

About 101% of the dose were recovered primarily in the feces within 7 days. The urinary recovery was only about 6-11% of the administered dose. About 93% of the administered dose were recovered with the excreta after 48 hr postdose. In bile duct-cannulated rats at 10 mg/kg (oral) dose, 98% of the administered radioactivity was recovered within 72 hours; 5-8% was excreted in the urine, 14-33% with the feces and 58-76% with the bile within 72 hours. The main elimination process appeared to be hepatic biotransformation and hepato-biliary transport. Cumulative excretion profile of radioactivity in urine and feces of male rats within 7 days after 10 and 100 mg/kg of radioactive ICL 670 is shown in the following table:

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Time Interval (hr)	Excretion in % of Administered Radioactivity			
	Urine		Feces	
	10 mg/kg	100 mg/kg	10 mg/kg	100 mg/kg
0-8	5.07	4.06	0.00	0.00
8-24	1.81	6.02	93.1	81.5
24-48	0.09	0.40	2.06	8.41
48-72	0.06	0.08	0.04	0.11
72-96	0.02	0.02	0.02	0.03
96-120	0.01	0.01	0.02	0.02
120-144	0.01	0.02	0.01	0.01
144-168	<0.01	<0.01	0.01	0.01

Cumulative excretion of radioactivity in urine, feces and bile of male bile duct-cannulated rats within 3 days after a single oral administration of 10 mg/kg of [¹⁴C]-ICL 670 are shown in the following table:

Time Interval (hr)	Excretion in % of Administered Radioactivity		
	Urine	Feces	Bile
0-24	5.65	14.6	68.5
24-48	0.55	6.25	0.70
48-72	0.09	0.68	0.08

Absolute Bioavailability of Three Different Oral Formulations in Dogs (Study No. R98-021)

Method: An absolute bioavailability study was performed in dogs to compare plasma profiles of three different oral formulations of ICL670 (two dispersible tablets with different excipients, forms A and B, and a dispersible sachet formulation, form C). The aim of the study was to collect *in vivo* performance data to help select the best formulation. An intravenous reference (form D) was used to determine the absolute bioavailability of the oral formulations. The following oral (p.o.) and intravenous (i.v.) formulations were administered to 8 male Beagle dogs with an interval of one week:

Form A: 1/2 dispersible tablet containing ϵ

1

Form B: 1/2 dispersible tablet containing ϵ

1

Form C: 2 sachets containing ϵ

1

Form D: 1.5 mL i. v. reference solution containing ζ

J

In this study, each animal received the same dose of 125 mg (11 mg/kg) p.o. and 15 mg (1.2 mg/kg) i.v. The concentrations of unchanged parent compound (ICL670) and its iron chelate CGP 82813 were determined in plasma samples taken up to 30 h post administration, using HPLC. Plasma levels of unchanged ICL670 was detected over at least 3 hours post i.v. administration and, and over at least 8 hours post oral dose.

Results: The mean plasma profiles appeared to be comparable for all three dispersible formulations: the C_{max} reached at 0.5 to 1 h post administration, the plasma levels declined to levels close to or below the lower limit of quantitation (LLQ = ζ J μ g/mL) at around 10 h post dose. Plasma levels of the iron chelate, CGP 82813, were low after oral administration, peaking around 3 to 4 h postdose. After i.v. administration, the chelate levels were below the LLQ. Following administration of either tablet or sachet formulation, pharmacokinetic parameters (T_{max} , C_{max} , and AUC) were in the same order of magnitude, and there was no apparent difference in absorption rate among three different formulations. The mean absolute bioavailability of the oral formulations A, B, or C versus the i.v. reference formulation D was similar and marginally above 100%. The individual absolute bioavailability ranged from 87 to 178% for formulation A, from 86 to 138% for formulation B, and from 91 to 204% for formulation C. The following table (from page of the study report) shows the results of this study.

Summary Table

T	Dose in mg/kg			t_{max} in h			C_{max} in μ g/mL			AUC in μ g-h/mL			f in %		
	Mean	Median	CV %	Mean	Median	CV %	Mean	Median	CV %	Mean	Median	CV %	Mean	Median	CV %
A	11.3	11.2	16	0.78	0.75	44	29.6	27.0	44	75.5	64.0	44	117	96	32
B	10.8	10.1	23	0.79	0.88	33	24.8	23.3	18	64.3	60.4	24	106	106	17
C	9.89	9.54	17	0.82	0.75	40	28.5	28.9	21	70.6	69.4	27	128	120	27
D	1.21	1.13	20				7.12	7.06	21	6.91	6.72	29			

T: treatment; Dose: actual dose; C_{max} : maximum plasma concentration observed at time t_{max} ; AUC: area under the plasma concentration-time curve extrapolated to time infinity; CV: coefficient of variation; f: absolute bioavailability in % of the i.v. reference form set to 100. C_{max} of the i.v. form D corresponds to C_{5min} .

Monkey**Absorption and Distribution in Male Marmosets**

Methods: Absorption was studied in male marmosets (3 animals/time point) using [¹⁴C]-labeled ICL 670 (specific activity 1.14 MBq/mg). The absorption, kinetics, biotransformation and excretion of ICL 670 were studied following i.v. (10 mg/kg) and p.o. (25 mg/kg) administration of radioactive ICL 670. Urine and feces were collected at time points ranging from 5 min to 168 h post-doses. Radioactivity in all samples was determined by liquid scintillation counting. Unchanged ICL 670 and its iron complex (CGP 82813) were determined in the plasma by an HPLC-UV detection method. Metabolite patterns in the plasma, bile, urine and feces were determined by HPLC with radioactive detection.

Results: After 10 mg/kg intravenous dose, the mean [¹⁴C] concentration in plasma at 5 minutes post dose was 75 μmol/L. Concentrations declined rapidly in a multiexponential manner and represented about 10% of the initial concentration at 8 hours post dose. The T_{1/2} was found to be 40-50 hours between day 1 and day 7. Following 25 mg/kg oral dose, C_{max} (56-127 μmol/L) in plasma was attained at 0.5-1.0 hours post dose. Thereafter, the radioactivity declined in a similar manner to that observed after intravenous dose and reached 10% of C_{max} at 24 hours post dose. The concentration of unchanged ICL 670 (determined by HPLC-UV method) at a faster rate than radioactive compound with a half-life of about 25 minutes after intravenous and oral dosing.

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The concentration of unchanged ICL 670 accounted for approximately 60% of that of total ICL 670 and 20% of that of ^{14}C labeled compound at 1 hour after intravenous and oral dosing. The concentration of Fe-ICL 670 complex was found to be very low ($<1\ \mu\text{mol/L}$).

Metabolism of [^{14}C]-ICL 670 in Male Marmosets

Methods: [^{14}C]-labeled ICL 670 (specific activity 1.14 MBq/mg) was administered in male marmosets (3 animals/time point) by i.v. (10 mg/kg) or p.o. (25 mg/kg) route. Urine and feces were collected at time points ranging from 5 min to 168 h post-dose. Radioactivity in all samples was determined by liquid scintillation counting. Unchanged ICL 670 and its iron complex (CGP 82813) were determined in the plasma by an HPLC-UV detection method. Metabolite patterns in the plasma, bile, urine and feces were determined by HPLC with radioactive detection.

Results: The biotransformation of ICL 670 was found to be extensive in male marmosets. More than 10 metabolites were identified in the feces and urine, most of which were found to be conjugates. The major compound identified in the plasma as ICL 670 and accounted for 35% and 23% of the total radioactivity in plasma based on the AUC (0-4h) for intravenous and the AUC (0-24h) for oral dose, respectively. Metabolite pattern in plasma after i.v. or p.o. was comparable. Two major metabolites were detected in plasma. In feces, 16 and 17% of the administered dose were represented by unchanged compound after i.v. and p.o., respectively. The two major metabolites represented 9-17% of the dose. Metabolism of ICL 670 took place in a route and dose-independent manner.

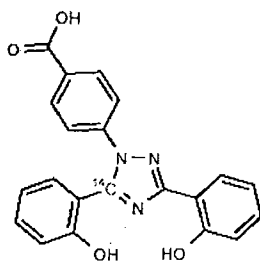
Excretion of Radioactivity in Urine and Feces in Male Marmosets

About 87-92% of the radioactivity was recovered with the excreta within 7 days. The majority of the administered dose was recovered in the feces and 5-22% of the dose was recovered in the urine. The excretion rate was most rapid during first two days, accounting for 76% of the administered dose. The excretion profile of radioactivity in the urine and feces after 10 (i.v.) and 25 (p.o.) mg/kg doses are shown in the following table:

Time Interval (hr)	Excretion in % of Administered Radioactivity			
	Urine		Feces	
	10 mg/kg (i.v.)	25 mg/kg (p.o.)	10 mg/kg (i.v.)	25 mg/kg (p.o.)
0-24	6.67	10.99	28.53	33.42
24-48	1.31	2.03	39.28	29.59
48-72	0.48	0.35	5.43	2.70
72-96	0.16	0.12	1.06	0.87
96-120	0.13	0.07	1.19	1.88
122-144	0.11	0.05	2.51	2.93
144-168	0.04	0.03	0.44	3.04

Absorption, Distribution, Metabolism and Excretion of ICL670 in Mice after Single Intravenous (10 mg/kg) and Oral (10 mg/kg and 300 mg/kg) administration of [^{14}C] ICL670 (Study No. R01-0012)

Methods: The aim of the study was to examine the disposition and metabolism of [^{14}C] ICL670 (specific activity = 0.093-1.862 MBq/mg) after a single intravenous and oral dose of 10 mg/kg and a single oral dose of 300 mg/kg to male and female mice. In this study, kinetics of radioactivity and the sum of free and iron bound ICL670 in the plasma (0.083, 0.5, 1, 2, 4, 8, 24, 72 h postdose), and excretion of radioactivity in the urine/feces (24 hours interval from Day 1-3) were determined. In addition, metabolite patterns and chemical structures of metabolites were also investigated in the plasma, urine and feces. Radioactivity was measured by using liquid scintillation counting (LSC) in the blood, plasma, urine, feces and HPLC/UV was used to determine the parent compound (sum of free and iron bound). HPLC combined with radioactivity detection was used for monitoring metabolite patterns. $\square$ **1** analysis was used for metabolites structure characterization. The following figure (from page 11 of the study report) shows the position of ^{14}C -radiolabeling.



Results:

Kinetics

After an i.v. dose of 10 mg/kg the kinetic parameters for ^{14}C concentrations in the blood and plasma were identical in male and female mice. The radioactivity declined rapidly in blood (to 10% of $C_{5\text{min}}$ within about 2 hours). After an oral dose of 10 mg/kg, the ^{14}C kinetics in the blood and plasma showed only small differences between male and females. The T_{max} of ^{14}C ranged from 30 min to 1 hour, indicating a rapid onset of absorption. After T_{max} , the radioactivity declined rapidly. After an oral dose of 300 mg/kg, the kinetics of radioactivity in the blood and plasma appeared to be different in males and females. In the females, the radioactivity declined by a factor of 200 between 8 and 72 hours. In the males, however, the radioactivity declined by a factor of 56-73 between 8 and 48 hours. The bioavailability at 10 mg/kg amounted to about 58% in male and female mice. The exposure in mice appeared to be over proportional at 300 mg/kg when compared to 10 mg/kg, oral dose (males: 7-fold higher; females: 4.5-fold higher).

Metabolism

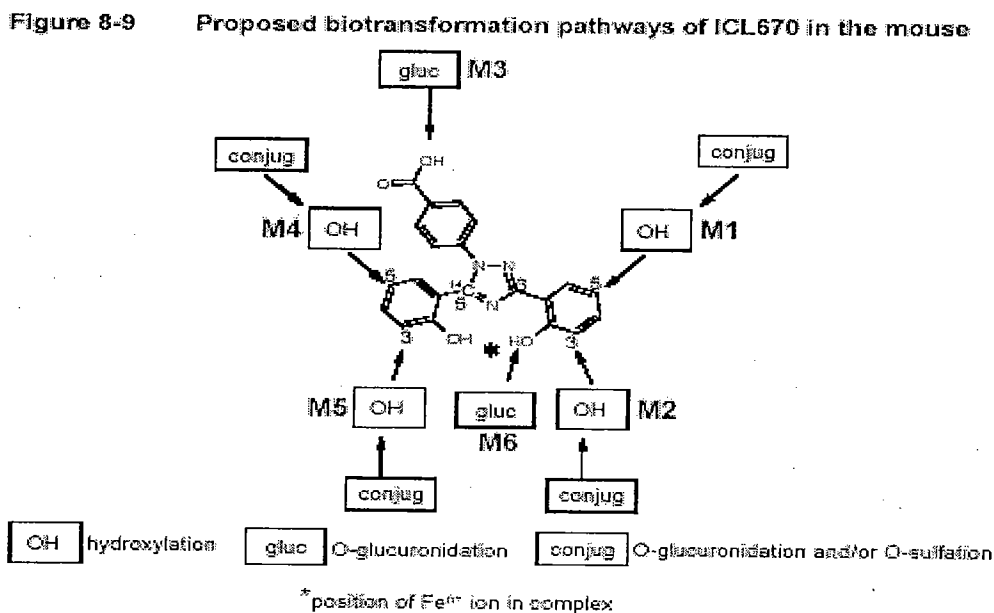
The following metabolites were identified in mice after i.v. and p.o. administration: M1 (hydroxylated), M2 and/or M4 (hydroxylated) and M5 (hydroxylated), M6 (glucuronidated), M3 (acyl glucuronidated). Unchanged ICL670 was the major compound in the systemic circulation (about 66-81% of the total administered radioactivity). M6 and M3 were only detected in systemic circulation, which accounted

for 1-5.8% and 2.9-13.3% of the exposure of the circulating drug, respectively. These two metabolites (M3 and M6) were excreted in the urine (2% of the administered i.v. dose). Irrespective of the dose and the gender the primary hydroxylated metabolites M1, M2 and/or M4 were detected in small amounts (< 6% of the dose) in the urine and feces of male and female mice after i.v. and p.o. administration. There were no apparent qualitative gender differences in metabolite patterns in excreta of mice at 300 mg/kg p.o. dose.

Excretion

After intravenous administration of 10 mg/kg, the radioactivity was completely excreted with feces and urine (4.7% of the administered dose) in male and female mice. About 90.7% and 66.9% of the administered dose was recovered in the feces and urine, respectively, within 72 hours post dose in both sexes following an oral dose of 300 mg/kg. At 72 hours post dose, 19.8% and 2.75% of the dose were found in the GI-tract and in the carcass of the males, respectively. The total recovery was 89.5% of the dose in males. At the 10 mg/kg, the excretion pattern was similar in male and female mice. At 300 mg/kg, the renal excretion was markedly more pronounced in males (18% of the dose) than in females (7% of the dose).

The following figure (from page 57 of the study report) shows the proposed biotransformation pathways of ICL670 in the mouse.



Relative Oral Bioavailability of Two Formulations (CSF and MF) of ICL670 in Dogs (Study No. R0101386)

Methods: This study was conducted to compare the pharmacokinetic (PK) properties of a new Market Formulation (MF, Form F) with that of the currently used Clinical Service Form (CSF, Form E). In this study, the pharmacokinetics of ICL670 were determined in dogs (n = 8) following a single dose of 125 mg/animal (about 12.5 mg/kg). The blood samples were collected at 0, 15, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hour post-dose. The samples were analyzed by HPLC-UV.

Results: Single oral doses of 125 mg ICL670 administered as a new potential Market Formulation (MF, test formulation) resulted in similar plasma concentration-time profiles as Clinical Service Form (CSF, reference formulation). The mean relative bioavailability of the test formulation was 113±13%. No significant differences in the PK parameters between the two oral formulations were observed. The following table shows the PK parameters (from page 6 of the study report).

Table 2-1 Mean pharmacokinetic parameters of total ICL670 in dogs

Pharmacokinetic parameters of total ICL670 (means ± SD) in plasma of male dogs after a single peroral administration of 125 mg ICL670 as formulation E (CSF) and F (MF) (cross-over, N=8).

Form	T _{max} [h] Median	C _{max} [µmol/L]	C _{max} /dose * [(µmol/L)/(mg/kg)]	AUC(0-24h) [(µmol/L)·h]	AUC(0-24h)/dose * [(µmol/L)·h / (mg/kg)]	f _{rel} (%)
E	1.25	57.3±18.3	4.79±1.53	217±75	18.1±6.3	100
F	1.25	62.1±14.9	4.47±0.98	259±47	18.7±3.6	113±36

* normalized to a dose of 1 mg/kg; see section 8.3

Comparative Mechanistic Transport Studies with NVPAML644-AB-1 (ICL670 Fe-Complex) and ICL670 Across Caco-2 cell Monolayers (Study No. R0201161)

Method: This study was conducted for transporter identification and comparative estimation of transporter affinity for ¹⁴C-ICL670 (specific activity = 1.928 MBq/mg) and ¹⁴C-NVP-AML644-AB-1 (specific activity = 1.696 MBq/mg), respectively, using human intestinal cell line Caco-2 grown on permeable filter support. The concentration dependent transport (1.562, 3.125, 6.25, 12.5, 25 and 50 µM) across Caco-2 cell monolayers was measured from the apical-to-basolateral as well as from the basolateral-to-apical side. At a concentration of 3.125 µM bidirectional studies were also conducted in the presence of the Pgp (P-Glycoprotein)/MRP1/MRP2 (multi drug resistant-related protein) inhibitor CsA (10 µM), Pgp/MRP1 inhibitor Verapamil (100 µM), the MRP1/MRP2 inhibitor Probenecid (50 µM) and the Pgp/MRP1/MRP2/BCRP (breast cancer resistant protein) inhibitor STI571 (50 µM), respectively. Radioactivity was measured using LSC.

Results: For NVP-AML644-AB-1 (1.562 µM) no transport, neither in apical-to-basolateral nor basolateral-to-apical direction, could be determined. All samples

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analyzed were below the LLQ of -- dpm . For ICL670 ($1.562 \mu\text{M}$), an apical-to-basolateral transport of about $6 \times 10^{-5} \text{ cm/min}$ was detectable within a time period of 120 minutes. Basolateral-to-apical transport on the other side occurred with a permeability value of about $37 \times 10^{-5} \text{ cm/min}$, which was significantly higher than the apical-to-basolateral transport. Addition of CsA, Probenecid and STI571 resulted in a significant increase of apical-to-basolateral and significant decrease of basolateral-to-apical transport of ICL670 ($3.125 \mu\text{M}$) with an average permeability value of about $20 \times 10^{-5} \text{ cm/min}$. However, the presence of verapamil did not appear to have any significant effect on ICL670 transport.

ICL transport through Caco-2 cells appeared to be dependent on the transport direction. Apical-to-basolateral transport was lower than basolateral-to-apical transport, suggesting that ICL670 was submitted to a prominent efflux system. Addition of the Pgp/MRP1 inhibitor, verapamil, did not increase apical-to-basolateral ICL670 permeation. However, in the presence of the Pgp/MRP1/MRP2 inhibitor (CsA), the MRP1/MRP2 inhibitor (probenecid) and the Pgp/MRP1/MRP2/BCRP inhibitor (STI571), respectively, a significant increase of apical-to-basolateral permeation was observed, indicating that the major efflux system involved in ICL670 transport is most likely MRP2. In contrast, no dependency on the transport direction was observed for NVP-AML644-AB-1. ICL670 was transported in a concentration-dependent manner across Caco-2 cells.

Overall, ICL670 was identified as a possible substrate for a prominent efflux system. Based on the results of this study, it appears that besides MRP2, other efflux transporter systems as e.g. BCRP may also be involved in the efflux of ICL670. The competitive inhibition potential of ICL670 was determined to be moderate-low ($K_m \cong 14 \mu\text{M}$). However, NVP-AML644-AB-1 was identified as a very low permeable compound. In contrast to ICL670 it could not clearly be identified to be a substrate for a prominent efflux system.

Mechanistic Transport Studies Across Caco- 2 Cell Monolayers (Study No. R0200436)

Methods: The objective of this study was to identify the prominent efflux system involved in the ICL670 transport and to determine the inhibitory potential of ICL670 on this transporter system. The concentration dependent transport (1, 5, 10, and $50 \mu\text{M}$) across Caco- 2 cell monolayers was measured from the apical (AP) to basolateral (BL) as well as from the basolateral to apical side. For $1 \mu\text{M}$ ICL670, apical to basolateral transport studies were also conducted in the presence of the potent efflux system inhibitors CsA ($10 \mu\text{M}$) and verapamil ($100 \mu\text{M}$), respectively.

Results: Apical to basolateral transport of ICL670 ($1 \mu\text{M}$, within a time period of 120 minutes) occurred with a permeability value of about $6 \times 10^{-5} \text{ cm/min}$. Basolateral to apical transport on the other side occurred with a permeability value of about $93 \times 10^{-5} \text{ cm/min}$, which was significantly higher than the apical to basolateral transport. This transport was concentration dependent. Addition of CsA or verapamil did not appear to result in a significant change of basolateral to apical transport of ICL670 indicating that the efflux system involved in ICL670 transport was neither Pgp nor MRP.

Investigation of Enterohepatic Circulation in Rats after Intravenous Administration of 10 mg/kg [¹⁴C]ICL670 (Study No. R0301123)

Methods: The objectives of the study were to characterize the extent of enterohepatic circulation of [¹⁴C]ICL670 (specific activity = 1.928 MBq/mg or 720 MBq/mmol) and its metabolites in male Wistar rats and the metabolite pattern in the bile of donor and receiver rats following a single i.v. dose of 10 mg/kg.

Group 1 (donor rats): Three bile duct-cannulated rats were administered 10 mg/kg of [¹⁴C]ICL670 intravenously. Bile, urine and feces were collected in 0-24 hour fractions (rat 1 and rat 2) or in 0-6 hour fractions (rat 3). From each individual fraction of bile and urine, aliquots of 50 µL were withdrawn and radioactivity was determined using LSC. Additionally, feces was collected and stored at -20°C until determination of radioactivity.

Group 2 (receiver rats): Three bile-duct cannulated rats were infused into the duodenum for 24 hours with a constant rate of 0.65 mL/h with about 15.5 g of the pooled bile (from Group 1). Following these 24 hours infusion, synthetic bile was infused into the duodenum with a rate of 1 mL/h for the next 24 hours. Bile, urine and feces were collected in 0- 24 hour and 24- 48 hour fractions. From each individual fraction of bile and urine, aliquots of 50 µL were withdrawn and radioactivity was determined using LSC. Bile fractions were stored at -20°C for analysis of the metabolite pattern. In addition, further determinations of radioactivity were conducted for feces, gastrointestinal tract, carcass and cage wash.

Results: After intravenous administration of 10 mg/kg [¹⁴C]ICL670 to bile duct-cannulated rats (Group 1, donor rats), the biliary recovery of radioactivity was about 78.2% within 24 hours post-dose and 61.5% within 6 hours post dose. In bile duct-cannulated rats (Group 2, receiver rats), which received the bile from the donor rats as an infusion into the duodenum, the total mean recovery of [¹⁴C] ICL670-related radioactivity in the bile, urine and carcass was 39.0% of the infused radioactivity. The rest of the infused radioactivity was recovered in the gastrointestinal tract, feces and cage wash. The total extent of enterohepatic circulation was determined to be about 30.5% of the i.v. dose.

Feasibility of the Dog as Food Effect Model of ICL670: Effect of Oil on Oral Bioavailability of Parent Drug and its Iron Chelate AML644 (Study No. R0301126)

Methods: This study was performed to assess whether Beagle dog is an appropriate model for assessment of the food effect of ICL670. In this study, three Groups (G0, GM and GF) of dogs (n = 9/groups) were administered a single oral dose 125 mg tablet/animal (about 12 mg/kg). A clinical tablet batch with 125 mg dosage strength was used in this study. The reference Group (G0) remained fasted until 6 h post dose. The treatment Group GM received 100 mL goat milk (3% fat) orally by gavage before tablet administration. The treatment group GF received 7.5 mL corn oil glycerides by gavage before oral administration of the tablet (approximately 6 g fat). Blood samples were

collected at 0 min (pre-dose), and 10, 30 min, 45 min, 1 h, 1.5, 2, 2.5, 3, 6, 8, 11, 24 h post-dose. Samples were analyzed by HPLC-UV method.

Results: When ICL670 was administered after fat (i.e. treatments GF and GM) the first concentration was quantifiable in the plasma 3 to 4 times later than after administration in fasted state. The peak level was obtained at 0.83h after administration when ICL670 was administered fasted whereas the peak level was obtained at 2.3 h and 2.6 h after administration when ICL670 was administered after pretreatment with oil-in-water emulsion and with oil, respectively. Administration of about 6 g fat as oral 'solution' had the same effects on PK parameters as 3% fat administered as milk (oil-in-water-emulsion). The mean dose related exposure to ICL670 was found to be in the same order of magnitude in all groups within the range of 66 to 69 $\mu\text{g}\cdot\text{hr}/\text{ml}$. However, compared to fasted state, the peak levels in the plasma were about 20% lower when fat was administered prior to ICL670. Peak level of AML644, the ICL670 iron complex, was reached 2 to 3-fold later when ICL670 was administered in fed state than when ICL670 was administered fasted.

Overall, administration of fat to dogs caused a shift of lag-time (3 to 4 times as compared to fasted values), and a shift of average T_{max} from $< 1\text{h}$ to $> 2\text{h}$. Exposure ($\text{AUC}_{0-\infty}$) of ICL670 remained similar in all treatment groups, whereas C_{max} dropped about 20% when fat was administered before dosing of ICL670. The mean ICL670 PK parameters in dogs are shown below (from page 12 of the study report).

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Table 3-1 Mean ICL670 PK parameters (%CV) after administration of oral ICL670 formulations to dogs

PK parameters	Treatment G_0 fasted dogs	Treatment G_F fed dogs 6 g fat as cornoil glycerides	Treatment G_M fed dogs 3g fat as goat milk
Actual dose [mg/kg]	11.9 (4)	11.9 (5)	11.9 (4)
AUClast [$\mu\text{mol}\cdot\text{h/L}$]	172 (24)	182 (23)	179 (20)
AUClast/dose, [($\mu\text{mol}\cdot\text{h/L}$)/(mg/kg)]	14.5 (23)	15.3 (23)	15.1 (17)
AUC(0- ∞) [$\mu\text{mol}\cdot\text{h/L}$]	177 (23)	188 (23)	186 (19)
AUC(0- ∞)/dose [($\mu\text{mol}\cdot\text{h/L}$)/(mg/kg)]	14.9 (22)	15.9 (22)	15.7 (16)
C _{max} [$\mu\text{mol/L}$]	57.8 (17)	43.4 (20)	44.1 (11)
C _{max} /dose [($\mu\text{mol/L}$)/(mg/kg)]	4.86 (16)	3.66 (21)	3.71 (9)
t _{max} [h]	0.83 (15)	2.6 (21)	2.3 (34)
t _{max} [h] range	0.75 to 1.0	1.5 to 3.0	1.0 to 3.0
t _{lag} [h]	0.24 (60)	0.97 (44)	0.69 (24)
t _{lag} [h] range	0.17 to 0.5	0.5 to 1.5	0.5 to 1.0
t _{1/2} [h]	2.3 (12)	2.0 (17)	2.3 (27)
f _{rel} [%] versus reference treat- ment G_0, based on AUClast	100 (by definition)	113 (35)	109 (31)
f _{rel} range	n.a.	52.5 to 166	71.5 to 150

Parameters obtained after single dose administration of 125 mg ICL670 as dispersible tablet to nine dogs per group, either fasted or fed before dosing; formulations applied, with subsequent rinse using 20 mL tap water; plasma concentration determined by ^1H NMR; AUC was calculated using the linear trapezoidal rule; CV: coefficient of variation; f_{rel} is the mean individual oral bioavailability as compared to the reference treatment G_0 set to 100% (based on mean dose-normalized AUClast of the reference treatment); t_{1/2} is the apparent half-life; n.a. = not available or not applicable.

Determination of Carrier-Mediated Cell Uptake of ICL670 via hOATP1, hOATP2, hOATP8 and hOCT1 using Pre-Injected *Xenopus oocytes* (Study No. R0400372)

Methods: The objective of this study was to identify the transporter system (s) involved in the carrier-mediated uptake of ICL670 and determination of the corresponding kinetic parameters (K_m and V_{max}) in *Xenopus laevis* oocytes expression system. Uptake mediated by the human Organic Anion Transporting Polypeptide 1 (hOATP1), hOATP2, hOATP8 or the human Organic Cation Transporter 1 (hOCT1) was measured by incubating the cRNA-injected transportocytes (T-oocytes) and water-injected control oocytes with different concentrations of ICL670 (2, 5, 10, 20, 50 and 100 μM) for 60 minutes in transport buffer at pH 7.4 at room temperature.

Results: A dose-dependent linear increase in ICL670 uptake was observed up to 100 μM concentration for the water-injected control oocytes. For all expression systems, uptake was not considered significantly different from uptake values into water-injected control oocytes indicating no affinity of ICL670 for these transporter systems. Uptake of positive control compound [^3H]E₁S (0.1 μM) into transportocytes overexpressing hOATP1, hOATP2 and hOATP8, respectively, was significantly increased (about 10-fold for hOATP2, about 3-to 4-fold for hOATP1 and hOATP8, respectively) in comparison to uptake into water-injected control oocytes. Similarly, uptake of [^{14}C]TEA (10 μM) into transportocytes overexpressing hOCT1 was increased by 4-fold in comparison to uptake into control oocytes (water-injected). Overall, based on the results of this study, ICL670 did not appear to be transported by hOCT1, hOATP1, hOATP2 or hOATP8.

2.6.4.4 Distribution

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Distribution**In Vitro Blood Distribution and Binding of ICL 670 to Plasma Proteins from Human, Dog, Rat and Marmoset**

The *in vitro* distribution of ICL 670, between erythrocyte and plasma, in human, dog, rat and marmoset blood was determined using [¹⁴C]-labeled ICL 670 and ultrafiltration technique. In human, the mean fraction in erythrocytes was 12.9 ± 4.4% for ICL 670 (5-100 µg/ml). A slight decrease (from 99.7% to 99.5%) in protein bound fraction was observed in plasma with increasing concentration of ICL 670 (7-166 µg/ml).

In rat blood, the mean fraction in erythrocytes was 19.0 ± 3.1% for ICL 670 (5-100 µg/ml). The protein bound fraction also decreased slightly from 5 to 100 µg/ml.

In dog blood, the mean fraction in erythrocytes was 15.1 ± 2.8% for ICL 670 (5-100 µg/ml). The protein binding (98.5%) remained constant with increasing concentrations of the drug (5-100 µg/ml).

In marmoset blood, the protein bound fraction remained constant (98.7%) with increasing concentrations of ICL 670 (10-100 µg/ml).

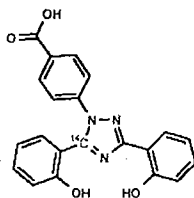
In Vitro Binding of ICL 670 to Albumin, α1-Acid Glycoprotein and γ-Globulins (Phase I Study)

The objective of the study was to determine the parameters of binding i.e. bound fraction, affinity constants and possibly number of sites to 3 purified proteins: albumin, α1-acid glycoprotein and γ-globulins. The binding studies were done using ultrafiltration and [¹⁴C]-ICL 670. ICL 670 was found to be highly bound to albumin (98-99%). The binding remained constant at 10 and 105 µg/ml of ICL 670. Analysis of the binding data revealed one saturable specific binding site and a non-specific binding site: $n_1 = 6.5$, $K_{a1} = 4.9 \times 10^4$ L/mol and $n_2K_{a2} = 9.4 \times 10^3$ L/mol, respectively. The binding to α1-acid glycoprotein decreased from 85 to 8% with increasing ICL 670 concentration from 0.5 to 105 µg/ml. Two classes of binding sites were observed in case α1-acid glycoprotein, one saturable site $n_1 = 0.7$ $K_{a1} = 4.3 \times 10^5$ L/mol and one non-saturable site $n_2K_{a2} = 5.4 \times 10^3$ L/mol. The fraction bound to γ-globulins appeared to be negligible.

Galactogenic Transfer, Kinetics and Metabolism in Milk and Plasma after Single Oral Administration of 10 mg/kg ¹⁴C-labeled ICL670 to Lactating Rats (Study No. R00-0015)

Methods: This study was conducted to assess the elimination of ICL670 into milk and kinetics of ICL670 and/or metabolites in the blood, plasma and milk after single oral (gavage, 10 ml/kg) administration to lactating rats at 10 mg/kg [¹⁴C]ICL670 (specific activity = 1.136 MBq/mg). The position of the radiolabeling (¹⁴C) is shown below (from page 6 of the report).

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Milk and blood samples were collected at 0.5, 2, 4, 8, 24, 48 and 72 hours post-dose. In Group 1 (N = 4), samples were taken at 0.5, 4, 24, 72 hours post-dose and in Group 2 (N = 4) samples were taken at 2, 8 and 48 hours post-dose. Radioactivity in the samples was measured by LSC. Samples were analyzed for metabolites using HPLC with radioactivity detection.

Results: The disposition of [^{14}C]ICL670 and/or metabolites in lactating rats following a single oral administration of 10 mg/kg [^{14}C]ICL670, was characterized by: 1) a rapid and extensive transfer of [^{14}C]ICL670 into the milk (3.3% of the administered dose within Day 1 post-dose), 2) the ratios ^{14}C concentrations in the milk/ ^{14}C concentrations in the plasma were 2.2, 11.6, 24.4, 16.6, 10.0, 1.1, 2.5 at 0.5, 2, 4, 8, 24, 48 and 72 h post dose, respectively, 3) about 80% of recovered radioactivity corresponded to [^{14}C] ICL670 and 4) no transfer of metabolites into the milk. These data suggested that ICL670 has the potential to be excreted through breast milk and nursing neonates might be exposed to ICL670 if the drug is administered to the nursing mother. This finding is important from the perspective of labeling.

Placental Transfer, Kinetics and Excretion of Radioactivity in Rabbits after Oral Administration of 50 mg/kg of [^{14}C]ICL670 (Study No. R0101631)

Methods: In this study, kinetics of [^{14}C]ICL670-related substances in the plasma, excretion of radioactivity in the urine and concentrations of radioactivity in the fetuses, were studied following an oral (gavage) dose of 50 mg/kg [^{14}C]ICL670 (specific activity = 17KBq/mg) to New Zealand rabbits (n = 3, GD17). Plasma samples were collected at 0.25, 0.5, 1, 2, 4, 8 and 24 h post-dose. The urine was collected during 0-24 h period. Fetuses were also examined for the radioactivity. Radioactivity was determined by LSC.

Results: The absorption appeared to be slow in the female rabbits. About 15% and 22% of the apparent C_{max} was attained at 30 minutes and 4 h post-dose, respectively. Excretion in the urine (0- 24 h) was about 9.91% of the administered dose was. At 24 h post-dose, mean ^{14}C concentration in the fetuses was about 0.26 nmol/g, i.e. about 60-fold lower (2% of the corresponding plasma of the dam) than the corresponding concentrations in the plasma of the dams (mean 15.6 $\mu\text{mol/L}$).

Tissue Distribution and Embryofetal Transfer in Pregnant Rats on Day 13 and Day 17 of Gestation Following a 30 mg/kg Oral Dose of [^{14}C]ICL670 (Study No. R0101632)

Methods: The objective of this study was to assess the tissue distribution and placental transfer of [¹⁴C]ICL670 and its metabolites in pregnant rats on GD13 and GD17 after a single oral dose of 30 mg/kg (10 ml/kg) of [¹⁴C]ICL670 (0.37 MBq/mg). Tissues (including blood) and whole body sections were collected at 1, 2, 8 and 24 post-dose. The radioactivity was measured using the LSC and quantitative whole body autoradioluminography (QWBAL) technique.

Results:

GD13: At 1, 8 and 24 h post-dose (p.d.), highest amount of radioactivity was found in the intestine, liver and kidney, and lowest tissue level was in the brain. Maximal levels were found at 2 h p.d. in all tissues except the intestine. Mean fetal levels of radioactivity was about 12-26 times lower than those of maternal blood, lower than those of all maternal tissues except brain (where the lowest tissue levels were found), and 3-36 times lower than those of the placentas. The radioactivity in the fetuses was about 0.005% of the administered dose at all time points, suggesting marginal transfer of drug-related material into the fetal compartment.

GD17: At all time points, blood levels of radioactivity were found to be higher than those in the tissues except bile, kidney, liver and adrenal (cortex). At 1, 2 and 8 h p.d., highest radioactivity levels were found in the bile, kidney and liver. Fetal levels of radioactivity were about 0.001% of the dose. Fetal levels of radioactivity were lower than those in the maternal blood and most maternal tissues and at least 3 times lower than those in the placenta and amnion up to 8 h p.d.

Overall, marginal transfer of drug-related material into the fetal compartment was observed on GD13 and GD17 (about 0.005% and 0.001% of the dose, respectively). The results of this study suggested that there may be some placental transfer of ICL670.

Distribution of Radioactivity in Sucking Rat Puppies and in the Dams after Single Oral Administration of 10 mg/kg ¹⁴C-Labeled ICL670 to Lactating Rats (Study No. R0200702)

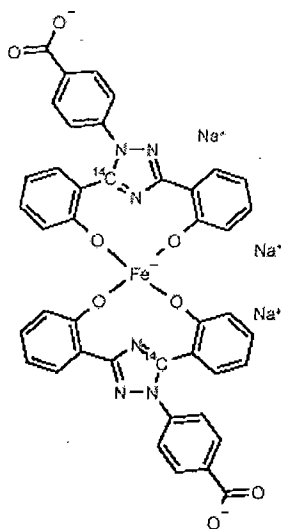
Methods: The objective of this study was to examine the distribution of ICL670 and its metabolites in sucking rat pups and in the lactating dams after single oral (gavage, 10 ml/kg) administration to the dams (n = 4) at 10 mg/kg [¹⁴C] ICL670 (5.1 MBq/mg). At 1, 2, 3, 4, 6, 8, 12 and 24 after the administration of ICL670 to the dams, one puppy each was sacrificed and prepared for quantitative whole body autoradioluminography (QWBAL). The dams were sacrificed at 24 h after the administration of ICL670 and prepared for QWBAL.

Results: Exposure to ICL670 and/or its metabolites was observed in the sucking puppies between 1 and 24 h following oral administration of 10 mg/kg [¹⁴C] ICL670 to lactating rats. However, ¹⁴C concentrations above the lower limit of quantitation (LLQ = 1 nmol/g) were detected only in the kidney cortex, liver, lung, muscle, esophagus, skin and

contents of stomach. C_{max} was observed at 8 hours post-dose in kidney cortex, liver, and lung and at 4 hours in the muscle. Generally the distribution pattern of radioactivity was similar in puppies and in dams. Overall, sucking puppies were exposed to ICL670 and/or its metabolites between one and at least 24 hours after oral administration of 10 mg/kg [¹⁴C] ICL670 to lactating rats.

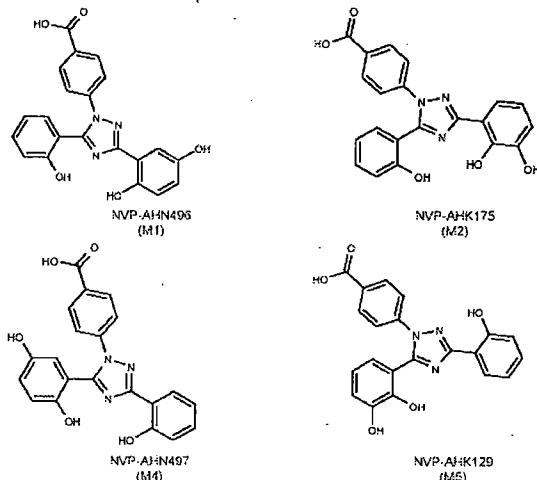
Disposition of [¹⁴C]AML644 (Iron Complex of ICL670) in Male Rats Following a Single Intravenous Dose of 10 mg/kg Labeled Compound (Study No. R0201158)

Methods: The objectives of the study were to characterize the disposition of [¹⁴C] AML644 (Iron complex of ICL670) in male Wistar rats (n = 4) following a single intravenous dose of 10 mg/kg [¹⁴C]AML644 (specific activity = 1.696 MBq/mg). The tissue distribution of radioactivity and the uptake of radioactivity into melanin-containing structures were also assessed in pigmented male Long Evans rats (n = 5) using QWBAL. Blood and plasma samples were collected at 0.083, 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h post-dose. Urine and feces were collected for a period of 24 h from Day 1-4. Tissues for QWBAL were collected at 0.083, 1, 8 and 24 h post dose (pigmented rats) and 168 h post-dose (pigmented and albino rats). The structure of the test compound is shown below (from page 7 of the study report).



The radioactivity in the blood, plasma and excreta pools was measured by liquid scintillation counting (LSC). Concentrations of AML644 in the plasma were quantified by τ 1 The tissue levels of radioactivity were determined by QWBAL. Metabolite patterns were determined by HPLC with radioactivity monitoring. Metabolite structures were characterized by τ j and by comparison with reference compounds (M1, M2, M4 and M5). The structures of these reference compounds are shown below (from page 10 of the study report).

3.2 Reference compounds



Results: After intravenous administration of 10 mg/kg [^{14}C] AML644 to male rats the ^{14}C concentrations in the blood declined rapidly and multiexponentially: 10%, 1%, 0.1% and 0.025% of $C_{5\text{min}}$ (mean 56.3 $\mu\text{mol/L}$) was reached between 1 and 2 h, at 8, at 48 and at 96 h, respectively. AML644 showed a low volume of distribution ($V_0 = 78 \text{ mL/kg}$) and a low plasma clearance ($\text{CL} = 3.92 \text{ mL/min/kg}$). Free, non-iron bound ICL670 was observed (4% of the AML644-concentrations) in the plasma at 5 min post-dose (p.d.). The concentrations of ICL670 were about 4 μM up to 1 h p.d. and then declined thereafter. At 2 h post-dose, the concentrations of AML644 and ICL670 appeared to be similar. All hydroxylated metabolites (M1, M2, M4 and M5), the O-glucuronide (M6) and the three sulfate conjugates (M7, M8 and M13) were excreted in the urine (no metabolite exceeded 1.3% of the dose).

The major component observed in the feces was AML644/ICL670 (40.5 % of the dose). All hydroxylated metabolites (M1, M2, M4 and M5) were detected in the feces after i.v. administration (about 24% of the dose with M1 and M2 being more prominent than M4 and M5). The tissue distribution of radioactivity was essentially intravascular up to 8 h p.d. Highest levels of radioactivity were found in the liver, bile, blood, kidney and adrenal. At 168 h p.d., residual radioactive traces in the kidney (cortex), bone marrow, spleen, liver and lung, were about 0.4% of the dose. No specific uptake of radioactivity into the melanin-containing structures and brain was observed. The administered dose was rapidly and completely recovered in the urine and feces within 96 h (95.4% of the dose). The urinary recovery was low (5.3% of the dose).

Comparative Analysis of *In Vitro* Blood Distribution and Plasma Protein Binding for [^{14}C]ICL670 and its Iron Complex [^{14}C]AML644 for Rat, Marmoset, Rabbit, Mouse, Dog and Human (Study No. R0201753)

Methods: The objectives of this study were to examine the *in vitro* blood distribution and plasma protein binding in the rat, marmoset monkey, rabbit, mouse, dog and human plasma (at 4, 20 and 80 µg/ml concentrations for both compounds) of [¹⁴C]ICL670 (specific activity = 1.977 MBq/mg) and its Fe³⁺ complex [¹⁴C]AML644 [Fe-(ICL670)₂] (specific activity = 1.696 MBq/mg). Gender differences were also examined for the mouse and the rabbit. For determination of the *in vitro* blood distribution, [¹⁴C]ICL670 and [¹⁴C] AML644 were incubated in the blood for 1 h at 37°C followed by separation of cell and plasma fraction by centrifugation. For determination of the *in vitro* plasma protein binding, test compounds were incubated in the plasma at 37°C for 1 h. Separation of the protein bound and free fraction was achieved by ultrafiltration. Total ¹⁴C radioactivity was quantified by ¹⁴C liquid scintillation counting (LSC). In addition, the interconversion between ICL670 and AML644 was also analyzed in a parallel study in the rat blood and plasma.

Results:

Blood distribution

At 4-80 µg/mL, both compounds were mainly distributed in the plasma fraction of the blood. In all species, the fraction in the plasma (Fp) appeared to be higher for the iron complex [¹⁴C]AML644 when compared to uncomplexed [¹⁴C]ICL670.

Blood distribution of [¹⁴C]ICL670 appeared to be species-dependent. In some species (male and female mouse, male and female rabbit and human) a trend for a decrease in Fp with increasing concentration was observed, which was not evident in the marmoset, dog and rat. The Fp's were as follows: human (94-95%) ≥ female rabbit (89-93%) ≈ dog (90-91%) > female mouse (79-85%) ≈ male rabbit (78-87%) ≥ marmoset (73-77%) ≈ male mouse (70-76%) ≥ rat (63-69%).

Plasma protein binding

Both compounds were extensively bound to plasma proteins at concentration range of 4-80 µg/mL. The unbound fraction (Fu) for [¹⁴C]AML644 appeared to be smaller compared to [¹⁴C]ICL670 in all the species tested. Plasma protein binding of [¹⁴C] ICL670 appeared to be species-dependent and there appeared to be a trend for increasing Fu with higher concentrations across all species except for marmoset. The following are the Fu's in different species: human (0.4-0.5%) < dog (0.6-0.7%) ≈ rat (0.7-1%) ≈ female rabbit (0.7-0.9%) ≤ female mouse (0.9-1.1%) ≈ male rabbit (0.9-1.2%) ≤ male mouse (1.1-1.6%) ≈ marmoset (1.4-1.8%). Plasma protein binding of [¹⁴C]AML644 was also species-dependent; a trend for increasing Fu with higher concentrations was evident in some species but not in the marmoset. The Fu's were as follows: dog (0.2%) ≤ human (0.3%) ≈ rat (0.3%) < female rabbit (0.6%) < male rabbit (0.8-0.9%) ≈ female mouse (0.9-1.0%) ≤ male mouse (0.9-1.1%) ≈ marmoset (0.9-1.2%).

Overall, the unbound fraction of the iron complex [^{14}C]AML644 compared to [^{14}C]ICL670 appeared to be smaller by a factor of 1.1-1.7 in the mouse, rabbit, marmoset and human, whereas the difference was about three-fold in the rat and dog. The species ranking for F_u 's for both ICL670 and its iron complex are as follows: human, rat, dog < rabbit, female mouse < male mouse, marmoset.

Disposition of [^{14}C]ICL670 in Wistar Rats and in Mrp2-Deficient (TR⁻) Rats Following A Single Intravenous Dose of 10 mg/kg (Study No. R0300259)

Methods: The objectives of the study were to characterize the disposition (pharmacokinetics in the blood/plasma, excretion balance, and metabolism) of [^{14}C]ICL670 (1.928 MBq/mg) in male Wistar rats (n = 6) and Mrp2 (Multidrug resistant protein 2)-deficient (TR⁻) rats (n = 6) following a single i.v. dose of 10 mg/kg. Blood and plasma samples were collected at the following time points: 0.083, 0.5, 1, 2, 4, 8, 24, 48, 72 h post-dose. Urine, bile and feces were collected for a period of 24 hour from Day 1-3. The radioactivity in the blood, plasma and excreta was measured by LSC. Plasma concentrations of ICL670 were quantified by []. Metabolite patterns were characterized by HPLC with radioactivity detection. Metabolite structures were characterized by []

Results:

After i.v. administration of 10 mg/kg of [^{14}C]ICL670 to Wistar rats, the ^{14}C concentrations in the blood and plasma declined rapidly and presumably in a bi-phasic manner. The half-lives were 0.8 h and 18 h during the early phase (0-4 h) and the later phase (8-72 h), respectively. After i.v. administration of the same dose to TR⁻ rats, the ^{14}C concentrations in the blood and plasma declined multi exponentially and notably slower than Wistar rats, which resulted in higher (5-fold) systemic exposure in TR⁻ rats than Wistar rats. However, the exposure to unchanged drug was comparable in both species.

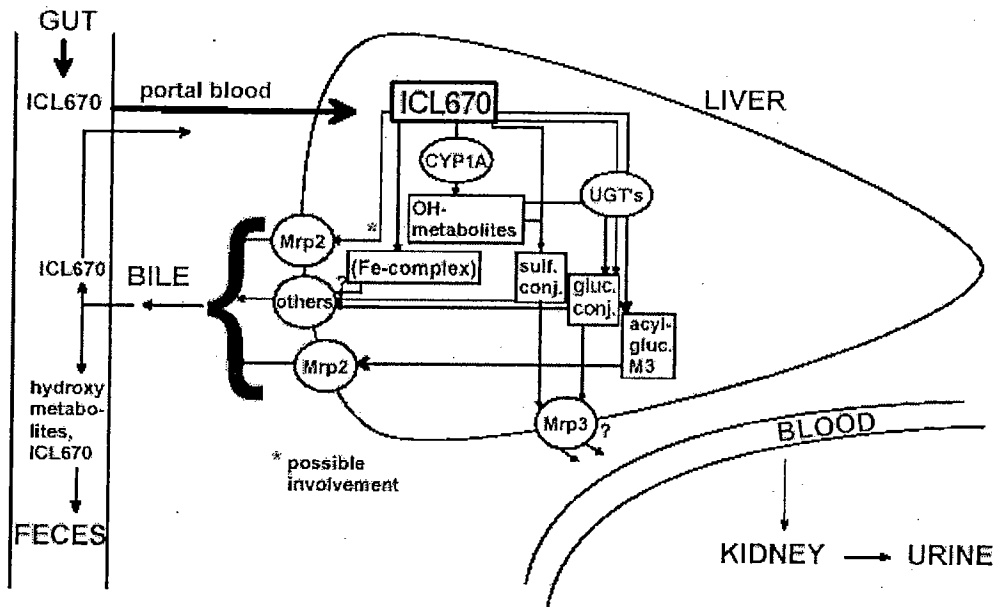
In Wistar rats, unchanged ICL670 (total of complexed and non-complexed) was the major compound identified in the systemic circulation following i.v. administration. The $\text{AUC}_{0.083-8\text{h}}$ of [^{14}C]ICL670 represented about 76% of the $\text{AUC}_{0.083-8\text{h}}$ of total radioactivity. The M3 was the only major metabolite detected in the plasma. In the systemic circulation of TR⁻ rats, unchanged ICL670 was also the major compound ($\text{AUC}_{0.083-8\text{h}}$ of [^{14}C] ICL670 represented about 23% of the $\text{AUC}_{0.083-8\text{h}}$ of total radioactivity). Generally, glucuronide and sulfate conjugates and hydroxylated ICL670 were observed in the TR⁻ rat plasma.

Biliary excretion of ICL670 and metabolites appeared to be considerably impaired in TR⁻ rats (from 81% in Wistar to 43% of the dose in Mrp2-deficient rats), however, the urinary excretion was higher in TR⁻ rats (from 7% in Wistar rats to about 33% in TR⁻ rats). In addition, a biliary excretion of 43% in the TR⁻ rats indicated that transporters other than Mrp2 might be involved in the biliary elimination of ICL670 and its metabolites as well.

The following metabolites were identified in both strains of rats: hydroxylated metabolites (M1, M2, M4 and M5), glucuronidation of the hydroxylated metabolite M4 leading to M6, glucuronidation of M1, M2, and M5 leading to M9, M10 and M11, respectively, acylglucuronidation leading to M3, sulfate conjugation of M1, M2, M5 and ICL670 leading to M7, M8, M11 and M13.

The biliary excretion of acylglucuronide M3 was considerably reduced in TR⁻ rats when compared to Wistar rats (3.3 vs. 30.5% of the dose), whereas amounts of sulfate conjugate in the bile of TR⁻ rats were increased (24.9% vs. 16.4% of dose).

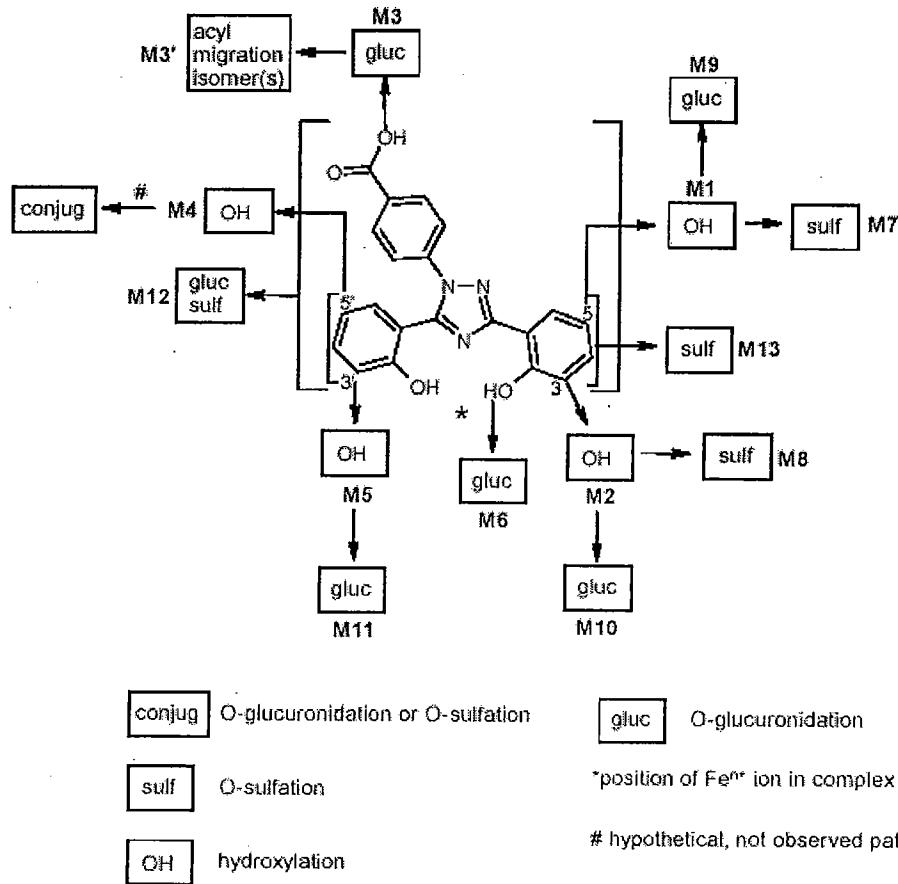
Overall, the results of this study indicated that Mrp2-mediated active transport might be involved in the biliary excretion of the acylglucuronide M3 (and possibly also of ICL670 itself). The disposition of ICL670 in rats and the role of Mrp2 transporter are summarized in the following diagram (from page 24 of the study report).



The following scheme (from page 47 of the study report) shows the possible pathways of biotransformation for ICL670 in rats.

Figure 9-7 Biotransformation of ICL670 in rats

The following scheme describes tentatively the biotransformation of ICL670 observed in rats.



2.6.4.5 Metabolism

In Vitro Biotransformation of ICL 670 by Liver Microsomes of Rat, Rabbit, Dog, Marmoset, and Man

Methods: Biotransformation of [¹⁴C]-ICL 670 was examined *in vitro* using liver microsomes from rat, rabbit, dog, marmoset, and man. Liver microsomes were prepared from frozen (dog and marmosets) and freshly removed (rat and rabbit) livers using homogenization and centrifugation techniques. Liver microsomes (0.5 mg protein/ml) then were incubated with radiolabeled ICL 670 at substrate concentrations ranging from 1-50 μmol/L in the presence of

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NADPH-regenerating system containing isocitrate-dehydrogenase (1 U/ml), NADP (1 mmol/L), isocitrate (5 mmol/L) and $MgCl_2$ (5 mmol/L). The samples were incubated at 37°C in a shaking water bath for up to 180 minutes. The reaction was stopped by adding ice-cold acetonitrile containing 1% (v/v) of trifluoroacetic acid (TFA) followed by centrifugation. The supernatant was withdrawn and analyzed by HPLC. The residual pellet was washed and dissolved by Soluene. The radioactivity of the dissolved pellet and the supernatant was determined by liquid scintillation counter. The pattern of the metabolites in the supernatant was determined by HPLC technique and supernatants from incubations with marmoset and human liver microsomes were subjected to ^{14}C analysis.

Results: The *in vitro* biotransformation by the microsomes of rat, rabbit, marmoset and man was found to be qualitatively comparable. Four major metabolite peaks (P3, P4, P5, and P6) were found in different ratios in rat, rabbit, marmoset and man. Two additional peaks (P1 and P2) were detected in rabbits and marmosets. In dog, the liver microsomes were not capable of metabolizing ICL 670. K_m and V_{max} values could not be determined because of the limited set of data. The highest initial velocity (V_0) of the overall [^{14}C]-ICL 670 disappearance was found to be 200-300 pmol/min.mg in marmoset and rat liver microsomes. This value was found to be lower in human (97 pmol/min.mg). ^{14}C analysis revealed that P3, P4, and P5 contained an additional oxygen atom compared to parent compound.

Characterization of Major Metabolites of ICL670 Formed in Rats and Marmosets after Single Oral and Intravenous Administration (Study No. R99-1771)

Methods: The aim of the present study was to characterize several metabolites involved in the biotransformation of ICL670. In the present study, the chemical structures of the relevant metabolites of ICL670 formed in the rat were elucidated. The metabolites were isolated from bile and feces collected in a previous ADME study [R97-522]. In Study R97-522, the rats had received single oral doses of 10 or 100 mg/kg of [^{14}C]ICL670, as discussed in detail before. Methods used for structure elucidation of metabolites included [^{13}C] ^1H -NMR (nuclear magnetic resonance), ^{13}C IMR, and ^{13}C NMR techniques. In previous ADME studies in marmosets [R98-315], metabolite patterns were observed, which were comparable to those for the rat. Several metabolites formed in the marmoset showed the same chromatographic and chemical properties as the metabolites formed in the rat.

Results: The major metabolite M3, in rats, mainly excreted through the bile, was identified as the acyl glucuronide of ICL670. M3 was also found to be a major metabolite in the marmosets. Hydroxylation was an important biotransformation pathway in rats and marmosets. Three primary, hydroxylated metabolites (M1, M2 and M4) from rat feces were identified by NMR. In both rat and marmoset, both M1 and M2 were formed by CYP1A. In the rat, but not in the marmoset, additional metabolites, M4 and M5, were formed by CYP2D. The metabolites were considered to have iron-binding capability. The combined metabolism results from this and from previous studies are summarized in the following figure (from page 36 of the study report).

Figure 13: Metabolite structures and percentage of dose of [14C]metabolites after p.o. administration to rats and marmosets

For rats, the percentages of the dose in bile, urine and feces are reported for bile-cannulated rats after 10 mg/kg [¹⁴C]ICL670 peroral dose [1]. For marmosets, the percentages of the dose in urine and feces after a 25 mg/kg [¹⁴C]ICL670 peroral dose are given [2]. In rat and marmoset plasma, the values at C_{max} are given.

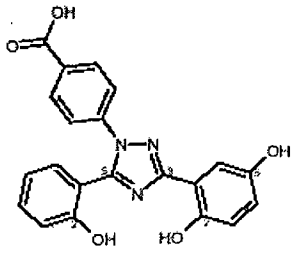
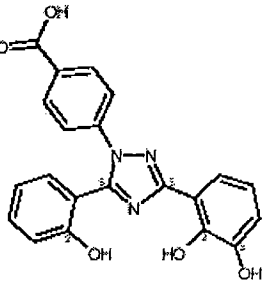
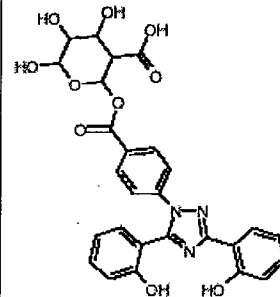
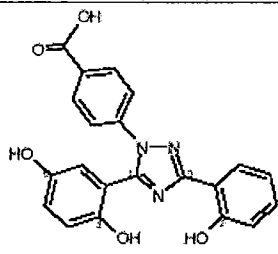
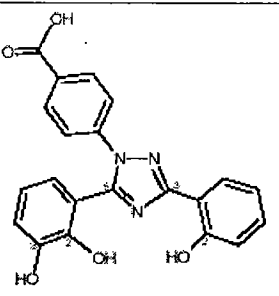
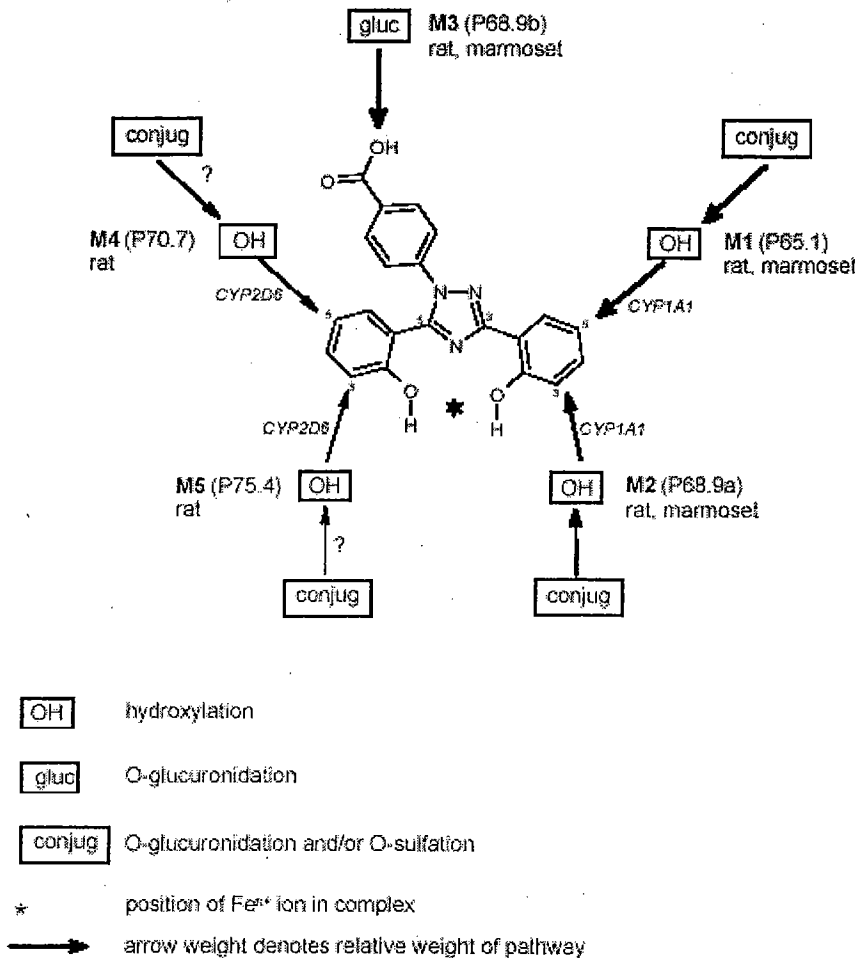
M1 (P65.1)	M2 (P68.9a)	M3 (P68.9b)
 rat plasma: - rat urine: 0.8% rat feces: 0.1% rat bile: 0.3% marmoset plasma: 0.5 μmol/L marmoset urine: 1.8% marmoset feces: 17.3% unstable at high pH/oxidizing conditions	 rat plasma: - rat urine: - rat feces: 0.1% rat bile: - marmoset plasma: - marmoset urine: - marmoset feces: 9.1%	 rat plasma: 0.1 μmol/L rat urine: 0.2% rat feces: - rat bile: 26.6% marmoset plasma: 28.6 μmol/L marmoset urine: 2.6% marmoset feces: - unstable at high pH (release of ICL670)
	M4 (P70.7)	M5 (P75.4)
	 rat plasma: 0.3 μmol/L rat urine: 1.6% rat feces: 0.1% rat bile: 2.8% marmoset plasma: - marmoset urine: 0.2% marmoset feces: 0.9% unstable at high pH/oxidizing conditions	 rat plasma: 0.1 μmol/L rat urine: - rat feces: 0.1% rat bile: 0.1% marmoset plasma: - marmoset urine: - marmoset feces: 0.9%

Figure 14: General biotransformation scheme of ICL670

Biotransformation pathways observed in rats and marmosets



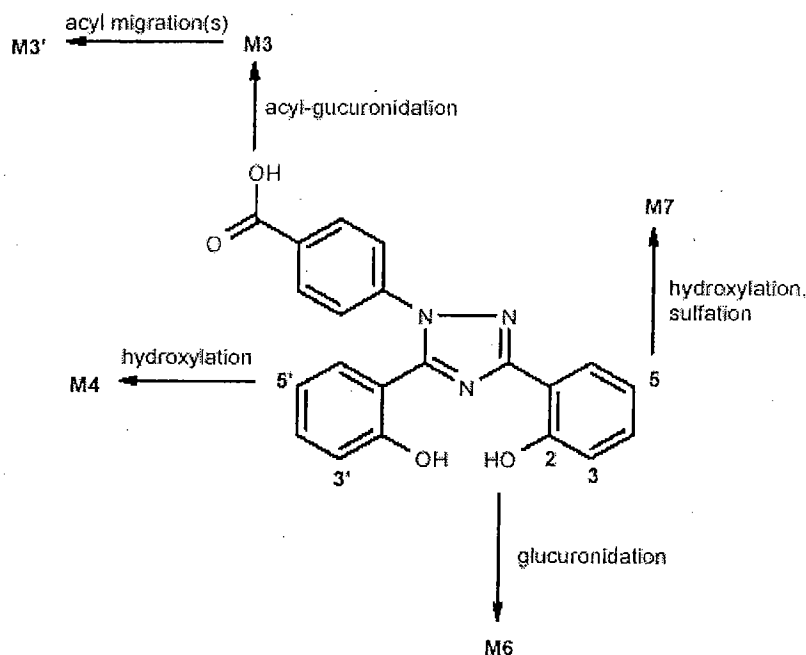
In Vitro Biotransformation of [¹⁴C]ICL670 in Hepatocytes from Rat and Human (Study No. R0201160)

Methods: The aim of this study was to examine the *in vitro* biotransformation of ICL670 in hepatocytes from rat and human especially the species dependent formation of the acyl-glucuronide M3. The biotransformation of [¹⁴C]ICL670 (specific activity = 2.002 MBq/mg) was examined in the rat and human hepatocytes. The incubations of [¹⁴C]ICL670 were performed in a serum-free culture medium at 37°C followed by centrifugation. The metabolite patterns were investigated by HPLC with radioactivity detection. The chemical structures of the metabolites were determined by C

Results: In human hepatocytes, [^{14}C]ICL670 was predominantly metabolized to the acyl-glucuronide (M3) by glucuronidation at the carboxylic acid moiety. In rat hepatocytes, the formation of the acylglucuronide (M3) was also observed. In both species additional metabolic reactions of [^{14}C]ICL670 were detected: (i) aromatic hydroxylation followed by subsequent sulfate conjugation resulting the metabolite M7 (5-O-sulfate), (ii) aromatic hydroxylation forming metabolite M4 (5'-hydroxy). However, phenolic glucuronidation with formation of metabolite M6 (2-O-glucuronide) was detected only in human hepatocytes. The following figure shows the proposed biotransformation pathways of ICL670 in rat and human hepatocytes (from page 21 of the study report).

Figure 8-5 Proposed biotransformation pathways of ICL670 in rat and human hepatocytes

The following scheme describes the proposed biotransformation pathways of [^{14}C]ICL670 observed *in vitro* in rat and human hepatocytes. Metabolite M6 was only observed in human hepatocytes. Whereas M3, M3', M4 and M7 were observed in both, in rat and human hepatocytes.



The following table shows the list of metabolites in rats and human hepatocytes (from page 15 of the study report).

Table 7-1 Mass spectral data of ICL670 and its metabolites in hepatocytes

data of incubates of [^{14}C]ICL670 (50 $\mu\text{mol/L}$) with human and rat hepatocytes for 3 h. The metabolites are listed in the order of elution from the HPLC

of 20 and 55 V (combined data); proposed structures shown in Figure 8-5; runs: ICL670_257, _258.

Components	Species ^{a)}	Observed ions (m/z) in runs ($^{12}\text{C}/^{14}\text{C}$)	
		M+H ⁺	Additional major signals
ICL670	r, h	374/376	346/348
M6	h	550/552	374/376 (M+H ⁺ -C ₆ H ₈ O ₆)
M7	r, h	470/472	390/392 (M+H ⁺ -SO ₂)
M4	r, h ^{b)}	390/392	b)
M3	r, h	550/552	374/376 (M+H ⁺ -C ₆ H ₈ O ₆)
M3'	r, h	550/552	374/376 (M+H ⁺ -C ₆ H ₈ O ₆)

a) Summarized data obtained from *in vitro* incubates of listed species:
r - rat, h - human.

b) Weak signal intensity

Determination of UDP Glucuronosyltransferase Isoenzymes Involved in the Metabolism of ICL670 (Study No. R0201162)

Methods: The present study was conducted to examine the uridine diphosphate glucuronosyltransferase (UGT) isoenzymes (UGT 1A1, UGT 1A4 and UGT 1A19) responsible for glucuronidation of [^{14}C]ICL670 (specific activity = 1.977 MBq/mg) as a part of its non-oxidative Phase II metabolism. In this study, non-oxidative [^{14}C]ICL670 metabolism due to (UGT) activity was examined *in vitro* using human liver microsomes. UGT-metabolism was also examined with a panel of recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) produced in insect cell membranes.

Results: In human liver microsomes, ICL670 was mainly metabolized by glucuronidation with the acylglucuronide being the major metabolite. Glucuronidation was found to be mediated predominantly by UGT1A1 and UGT1A3 (accounting together for about 95% of the glucuronide formation) with minor contributions of UGT1A7 and UGT1A9. Only trace activities were observed with UGT1A4, UGT1A6, UGT1A8, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17.

Analysis of the Interconversion Between ICL670 and its Fe³⁺ Complex AML644 in Rat Blood and Plasma (Study No. R0201163)

Methods: This study was conducted to quantify the interconversion between ICL670 and its Fe³⁺ complex, AML644, in the male rat blood and plasma at two different concentrations (4 and 80 µg/ml for both ICL670 and AML644). The incubation times in the plasma were as follows: 0, 1, 3, 8 and 24 h. The incubation times in the blood were as follows: 0.5, 1, 3, 8 and 24 h. The samples were analyzed following protein precipitation by [] method.

Results:

Incubation in the plasma:

AML644 was converted into ICL670 in the rat plasma at 37°C. This was found to be more pronounced at the lower concentration. About 60% of AML644 was converted in 8h at 4 µg/mL concentration and about 13% conversion was seen at the high concentration (80 µg/mL).

Incubation in the blood:

AML644 converted into ICL670 in the rat blood at 37°C. This was more pronounced at the lower concentration. About 57% of AML644 was converted in 8h at 4 µg/mL and about 34% conversion was observed at 80 µg/mL.

Assignment of Metabolites of ICL670 in the Rat, Mouse, Marmoset and Human ADME Studies (Study No. R0400128)

Methods: In previous metabolism studies with [¹⁴C]ICL670 in the rat (R97- 522) and marmoset (R98- 315), metabolite peaks observed in the plasma, urine, bile and feces extract could not be completely assigned to defined metabolite structures. Complete structure elucidation was hampered partially by insufficient sensitivity of the mass spectrometric methods. Later, more sophisticated analytical techniques (various NMR techniques, [] etc.) were used for identifying the metabolites. In this report, metabolite peaks observed in previous studies were assigned to the well characterized metabolites across all tested species including man to facilitate a comparison of the results from older studies with those from recent investigations. For this purpose some old samples were reanalyzed by the new methods.

Results: Across all animal and human ADME studies, apart from unchanged ICL670, the following 13 metabolites (shown in the following table from page 6 of the study report) of ICL670 were identified *in vivo* in the plasma and excreta of humans, marmosets, rats and mice.

Table 3-1 Peak identification and metabolite and names

Metabolite identification	Metabolite name
Peak identification in [DMPK(CH) R97-522] and [DMPK(CH) R98-315]	
M1	P65.1 5-hydroxy metabolite of ICL670
M2	P68.9(a) ¹ 3-hydroxy metabolite of ICL670
M3	P68.9(b) ² Acyl glucuronide of ICL670
M4	P70.7 5'-hydroxy metabolite of ICL670
M5	P75.4 3'-hydroxy metabolite of ICL670
M6	P54.3 2-O-glucuronide metabolite of ICL670
M7	P49.7 5-O-sulfate metabolite of ICL670
M8	P48.8 3-O-sulfate metabolite of ICL670
M9	P46.2 5-O-glucuronide metabolite of ICL670
M10	- 3-O-glucuronide metabolite of ICL670
M11	- 3'-O-glucuronide metabolite of ICL670
M12	- 2- or 2'-O-sulfate of an O-glucuronide of ICL670
M13	- 2- or 2'-O-sulfate metabolite of ICL670

¹ P68.9 in [DMPK(CH) R97-522], P68.9a in [DMPK(CH) R98-315]

² P68.9 in [DMPK(CH) R97-522], P68.9b in [DMPK(CH) R98-315]

Comparative Systemic Exposure to ICL670, Iron-Complexed ICL670 and 13 Metabolites of ICL670 Across Species Including Man (Study No. R0400311)

In this report, the systemic exposure data (AUC values) for humans and different animal species were compiled for comparison of exposure across species. The most relevant compounds to be considered for assessment of the systemic exposure were unchanged ICL670, the iron complex AML644 and the main metabolite M3 (acyl glucuronide; AcylGluc-ICL) in the plasma. Additional 12 metabolites of ICL670 detected *in vitro* in human liver microsomes and/or *in vivo* in the plasma and excreta of human, marmoset, rat and mouse were also listed. The therapeutic dose level in the humans was also compared with NOAELs in animals. Safety factors were calculated based on the AUC values obtained at respective NOAELs. Additionally, a qualitative overview of all metabolites characterized *in vitro* in the human liver microsomes and *in vivo* in plasma and excreta of human, marmoset, rat and mouse were also provided.

Clinical data were available from studies C1CL670 101 and C1CL670 104, and from the ADME study C1CL670 A0115. The proposed therapeutic dose ranges from 10 to 20 mg/kg/day. The plasma AUC values were obtained from the study C1CL670 104 at 1000 mg/day or 20 mg/kg/day (multiple doses) and were used for across-species comparison. Approximately 91% of total ¹⁴C in the plasma was found to be due to ICL670. The iron complex AML644 accounted for about 10%, and metabolite M3 (acyl glucuronide metabolite of ICL670) accounted for 3%. No other metabolites were detected in the human plasma.

Preclinical metabolite data were available from ADME studies in rats (R97-522, R0201158, R0300259 and R0301123), marmosets (R98-315), and mice (R01-0012). It is to be mentioned here that these animals were not iron-overloaded. Toxicokinetics data

were available from toxicity studies in non iron-overloaded rats (987037), marmosets (982027) and iron-overloaded mice (981060). Apart from unchanged ICL670, 13 metabolites of ICL670 were identified *in vitro* in the human liver microsomes and *in vivo* in the plasma and excreta of human, marmoset, rat and mouse as shown in the following table (from page 6 of the previous study report R0400128).

Table 3-1 Peak identification and metabolite and names

Metabolite identification		Metabolite name
Peak identification in [DMPK(CH) R97-522] and [DMPK(CH) R98-315]		
M1	P65.1	5-hydroxy metabolite of ICL670
M2	P68.9(a) ¹	3-hydroxy metabolite of ICL670
M3	P68.9(b) ²	Acyl glucuronide of ICL670
M4	P70.7	5'-hydroxy metabolite of ICL670
M5	P75.4	3'-hydroxy metabolite of ICL670
M6	P54.3	2-O-glucuronide metabolite of ICL670
M7	P49.7	5-O-sulfate metabolite of ICL670
M8	P48.8	3-O-sulfate metabolite of ICL670
M9	P46.2	5-O-glucuronide metabolite of ICL670
M10	-	3-O-glucuronide metabolite of ICL670
M11	-	3'-O-glucuronide metabolite of ICL670
M12	-	2- or 2'-O-sulfate of an O-glucuronide of ICL670
M13	-	2- or 2'-O-sulfate metabolite of ICL670

¹ P68.9 in [DMPK(CH) R97-522], P68.9a in [DMPK(CH) R98-315]

² P68.9 in [DMPK(CH) R97-522], P68.9b in [DMPK(CH) R98-315]

The following table (from page 6 and 7 of the study report) shows the systemic exposure (AUC) to ICL670, AML644 and acyl glucuronide metabolite M3 across species observed in ADME studies.

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Table 2-2 Raw data compilation: Systemic exposure to ICL670, iron-complexed ICL670 and the acyl glucuronide metabolite M3 of ICL670 in humans, marmosets, rats and mice

Compound/metabolite	Human	Marmoset ^f	Rat ^f	Rat ^f	Mouse ^{f,i}	Mouse ^{f,j}
	male & female	male	male	male	male & female	male & female
	1000 mg/day 20 mg/kg/day, MD	25 mg/kg, SD	10 mg/kg, SD	100 mg/kg, SD	10 mg/kg, SD	300 mg/kg, SD
	AUC (µmol·h/L)	AUC (µmol·h/L)	AUC (µmol·h/L)	AUC (µmol·h/L)	AUC (µmol·h/L)	AUC (µmol·h/L)
ICL670	1404 ^{a,k} / 688 ^h	n.a.	20.4 ^f	2160 ^f	n.a.	n.a.
ICL670 Fe-Complex	81 ^{a,k} / 79 ^h	n.a.	6.7 ^f	81.3 ^f	n.a.	n.a.
ICL670 + Fe-Complex	1485 ^a / 767 ^h	218 ^c	27.1 ^f	2251 ^f	33.7 ^{f,i}	5924 ^{d,i}
M7 (5-O-Sulf-ICL)	n.d.	73 ^k	0.1 ^g	n.d.	n.d.	n.d.
M8 (3-O-Sulf-ICL)	n.d.	73 ^k	n.d.	n.d.	n.d.	n.d.
M5 (2-O-Gluc-ICL)	n.d.	17	n.d.	n.d.	2.1 ^{f,i}	503 ^{d,i}
M1 (5-HO-ICL)	n.d.	0.5	n.d.	n.d.	n.d.	n.d.
M5 (3'-HO-ICL)	n.d.	n.d.	0.1 ^g	6.1 ^g	n.d.	n.d.
M3 (AcylGluc-ICL)	27 ^{b,h}	204 ^c	1.1 ^g	42.6 ^g	5.4 ^{f,i}	1607 ^{d,i}
[¹⁴ C]ICL670	710 ^{a,h}	171 ^c	31.7 ^f	2185 ^f	35.0 ^{f,i}	6634 ^{d,i}
Reference:	[0101] & [0104] & [0115]	[R98-315] [R99-1771]	[R97-522] [R99-1771]	[R97-522] [R99-1771]	[R01-0012]	[R01-0012]

Legend to Table 2-2:

- n.a.: not assessed;
- n.d.: not detected;
- ^a: AUC(0-inf) or AUC_∞ at steady-state;
- ^b: AUC(0-inf) obtained from [¹⁴C]AUC(0-24h) multiplied by a factor of 1.7 ([¹⁴C]AUC(0-inf) / [¹⁴C]AUC(0-24h));
- ^c: AUC(0-72h) obtained from ICL670-AUC(0-24h) multiplied by a factor of 1.5 (=AUC(0-72h) / AUC(0-24h));
- ^d: AUC(0-72h);
- ^e: data from multiple dose trial [Clin. Study C1CL670 0104];

^f: AUC(0-24h);
^g: AUC(0-8h);
^h: data from human ADME trial [Clin. Study C1CL670 A0115];
ⁱ: AUC value represents mean over males and females;
^j: marmosets, rats and mice were not iron-overloaded;
^k: M7 and M8 appear in the radiochromatogram as a double peak (peak sum 90 µmol·h/L) with a peak-ratio of about 1:1.

The following table (from page 8 of the study report) shows the systemic exposure (AUC) to ICL670 at NOAEL in different species.

Table 2-3 Raw data compilation: Systemic exposure to ICL670 (sum of ICL670 + iron-complexed ICL670) in the toxicity species

The AUC values represent overall means of the first dose and repeated dose values.

Species (Duration of treatment)	Doses (mg/kg/day)	Sampling period (day/week)	AUC _(0-24h)			C _{max}			Ref.
			male (µmol-h/L)	female (µmol-h/L)	overall mean (µmol-h/L)	male (µmol/L)	female (µmol/L)	overall mean (µmol/L)	
Marmoset ^a (39 weeks)	40 (NOAEL)	Day 1	164	185		32.7	37.0		[982027]
		Week 39	186	245	198	41.5	34.6	36.4	
Rat ^b (26 weeks)	30 (NOAEL)	Day 1	98.0	165.8		19.5	72.3		[987037]
		Week 12	256.3	158.8		64.9	64.5		
		Week 25	164.4	55.7	150	68.3	20.1	54.9	
Mouse ^b (4 weeks)	100 (NOAEL)	Day 2	581	570		145	168		[981060]
		Week 4	549	396	525	169	115	154	

^a: rats and marmosets were not iron-overloaded;

^b: the mice were given iron-supplemented food (iron-overloaded).

The following table shows the comparative systemic exposure to ICL670, AML644 and different metabolites across species (from page 9 and 10 of the study report) from ADME studies.

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Table 2-4 Comparative systemic exposure to ICL670, iron-complexed ICL670 and the glucuronide metabolite M3 of ICL670 across species including man - normalized to AUC at NOAEL.

Comparative systemic exposure to ICL670, iron-complexed ICL670 and its metabolites in plasma, after single (SD) and multiple (MD) oral administration of ¹⁴C-labeled and non-radiolabeled ICL670 to humans, marmosets, rats and mice. Exposure multiples (*SF*, safety factors; given *italic* in brackets) are expressed as the ratio of the exposure (normalized to AUC at NOAEL) of ICL670, iron-complexed ICL670, and metabolites of ICL670 observed in the animal studies versus the systemic exposure thereof in humans after multiple dosing of daily oral doses of 1000 mg ICL670. Where not stated otherwise, the animals were not iron-overloaded. The raw data are listed in Table 2-2. NOAEL reference values (bolded) were from Table 2-3.

Compound / metabolite	Human	Marmoset	Rat	Rat	Mouse	Mouse
	male & female	male	male	male	male & female	male & female
	1000 mg/day (20 mg/kg/day, MD)	Data normalized to NOAEL: 40 mg/kg (Data basis: 25 mg/kg, SD)	Data normalized to NOAEL: 30 mg/kg (Data basis: 10 mg/kg, SD)	Data normalized to NOAEL: 30 mg/kg (Data basis: 100 mg/kg, SD)	Data normalized to NOAEL: 100 mg/kg (Data basis: 10 mg/kg, SD)	Data normalized to NOAEL: 100 mg/kg (Data basis: 300 mg/kg, SD)
	AUC ^a (µmol·h/L)	AUC _{normalized} (µmol·h/L)	AUC _{normalized} (µmol·h/L)	AUC _{normalized} (µmol·h/L)	AUC _{normalized} (µmol·h/L)	AUC _{normalized} (µmol·h/L)
ICL670	1404 ^{a,b} / 688 ^{a,b}	n.a.	113 (0.08)	144 (0.10)	n.a.	n.a.
ICL670 Fe-Complex	81 ^c / 79 ^a	n.a.	37 (0.48)	6.1 (0.08)	n.a.	n.a.
ICL670 + Fe-Complex	1485 ^c / 767 ^c	198 (0.13)	150 ^b (0.10)	150 ^b (0.10)	525 ^b (0.35)	525 ^b (0.35)
M7 (5-O-Sulf-ICL)	n.d.	66 (n.c.) ^f	0.6 (n.c.)	n.d.	n.d.	n.d.
M8 (3-O-Sulf-ICL)	n.d.	66 (n.c.) ^f	n.d.	n.d.	n.d.	n.d.
M8 (2-O-Gluc-ICL)	n.d.	16 (n.c.)	n.d.	n.d.	34 (n.c.)	48 (n.c.)
M1 (5-HO-ICL)	n.d.	0.4 (n.c.)	n.d.	n.d.	n.d.	n.d.
M5 (3'-HO-ICL)	n.d.	n.d.	0.6 (n.c.)	0.4 (n.c.)	n.d.	n.d.
M3 (AcylGluc-ICL)	27 ^a	185 (0.9)	6.1 (0.23)	2.8 (0.11)	87 (3.2)	155 (5.7)
[¹⁴ C]ICL670	710 ^d	155 (0.22)	176 (0.25)	146 (0.21)	596 (0.84)	628 (1.2)
Reference	[0104] & [0110]	[R98-315]	[R97-522]	[R97-522]	[R01-0012]	[R01-0012]

Legend to Table 2-4:

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- n.a.: not assessed;
- n.c.: not calculable;
- (,): Ncific values in brackets means safety factors of systemic exposure;
- a.: AUC(0-inf) or AUC_τ at steady-state;
- b.: overall AUC value at NOAEL;
- c.: from [Clin. Study C1CL670 0104];
- d.: from [Clin. Study C1CL670A0115];
- e.: AUC values are means over male and females;
- f.: M7 and M8 appear in the radiochromatogram as a double with a peak-ratio of about 1:1.

The following table (from page 11 and 12 of the study report) shows the across species correlation of ICL670 and its metabolites observed *in vitro* in human liver microsomes and *in vivo* in plasma and excreta of human, marmoset, rat and mouse.

Table 2-5 Across species correlation of ICL670 and its metabolites observed *in vitro* in human liver microsomes and *in vivo* in plasma and excreta of human, marmoset, rat and mouse

Presence (t: trace, +: minor, ++: substantial, +++: prominent) or absence (-) of ICL670 and its metabolites identified *in vitro* in human liver microsomes of as well as *in vivo* in plasma and excreta of human, marmoset, rat and mouse.

Parent	ICL670	Human	Human			Marmoset			Rat				Mouse			
		<i>in vitro</i> liver micro- somes	<i>in vivo</i> 1000 mg, p.o.			<i>in vivo</i> 25 mg/kg, p.o.			<i>in vivo</i> 10 mg/kg, p.o.				<i>in vivo</i> 300 mg/kg, p.o.			
			Pl	Ur	Fec	Pl	Ur	Fec	Pl	Ur	Fec	Bile	Pl	Ur	Fec	
Parent	ICL670	+++	+++	+	+++	+++	++	++	+++	+	+++	++	+++	+	+++	
Phase I																
M1	6-hydroxy ICL	++	-	-	+	t	++	++	-	+	++	t	-	+	+	
M2	3-hydroxy ICL	++	-	-	-	-	-	++	-	++	+	-	-	+	+	
M4	5'-hydroxy ICL	++ ^a	-	-	+	-	+	-	-	-	+	+	-	+	+	
M5	3'-hydroxy ICL	+ ^b	-	-	-	-	-	-	t	-	t	t	-	-	t	
Phase II (Conjugates)																
M3	AcylGluc-ICL isomers	-	+	+	-	+++	++	-	+	+	-	+++	+	+	-	
M6	2-O-gluc-ICL	-	-	+++	-	+	+	-	-	++	t	+	t	+	-	
M7	5-O-sulf-ICL	-	-	+	+	++	+	t	t	t	-	++	-	++	-	
M8	3-O-sulf-ICL	-	-	-	-	++	+	t	-	++	-	++	-	++	-	
M9	5-O-gluc-ICL	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
M10	3-O-gluc-ICL	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
M11	3'-O-gluc-ICL	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
M12	(2- or 2'-O-sulf)-O-Gluc-ICL	-	-	-	-	-	-	-	-	-	-	t	-	-	-	
M13	(2-O or 2'-O-sulf)-ICL	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
Reference->		[R09-03E]	[C1CL670A0115]			[R09-315] [R09-1771]			[R07-522] [R0300266] [R0201186] [R0301123] [R09-1771]				[R01-0012]			

Legend to Table 2-5

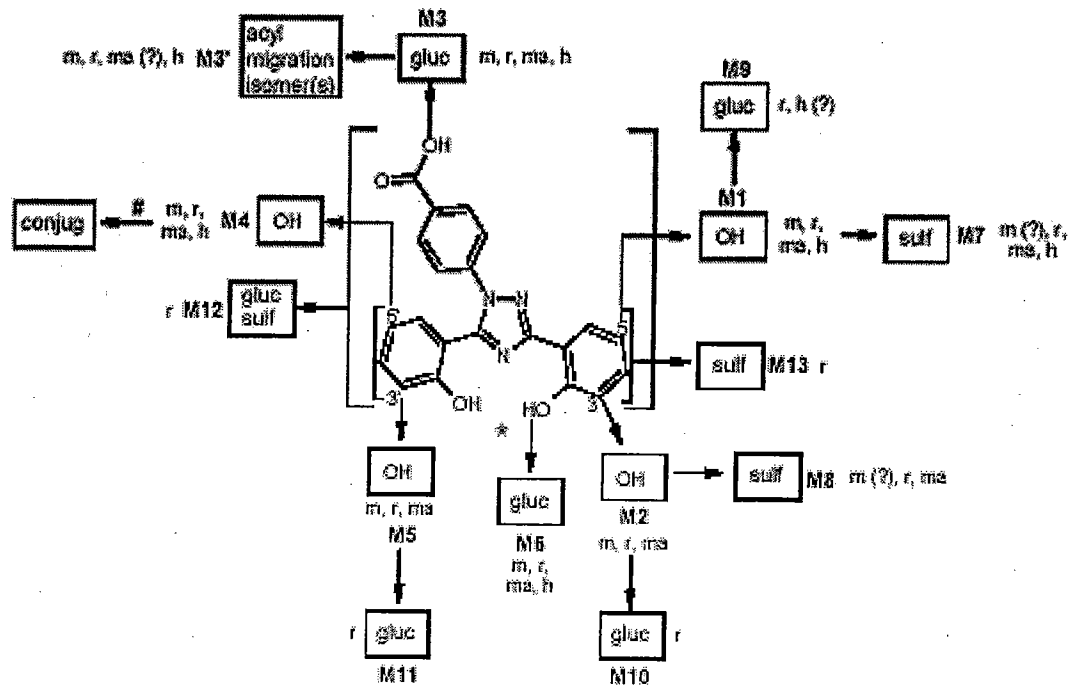
Pt:	Plasma;
Ur:	Urine;
Fec:	Feces;
+++:	prominent component : >40-99% of total ^{14}C in biological matrix;
++:	substantial component : >10-40% of total ^{14}C in biological matrix;
+	minor component : >2-10% of total ^{14}C in biological matrix;
t	trace component : $\leq$ 0.1-2% of total ^{14}C in biological matrix;
x	not detected;
*	in Report [R09-078] the metabolites M4 and M6 are designated as M3 and M4, respectively.

The proposed metabolic pathways of ICL670 in the human, marmoset, rat and mouse are shown in the following diagram (from page 13 of the study report).

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Figure 2-1 Metabolic pathways of ICL670 in human, marmoset, rat and mouse

Figure taken from [R0400128]



Preliminary Evaluation of an *In Vitro* Reporter Gene Assay for Measurement of PXR-Mediated Induction of Drug Metabolizing Enzymes (Study No. R0400619)

Methods: This study was conducted to examine the potential of ICL670 to induce CYP3A4, an important inducible enzyme, which is involved in the metabolism of many drugs. Induction of CYP3A4 can lead to an increased clearance of drugs which are also metabolized by this isoenzyme. The expression of CYP3A4 was considered to be regulated mainly by the ligand-activated transcription factor pregnane X receptor (PXR). In this study, the sponsor measured CYP3A4 induction by using the use of reporter gene cells. DPX2 is a CYP3A4 reporter gene cell line made by stable transfection of a reporter gene construct into the human hepatoma cell line HepG2. The reporter gene

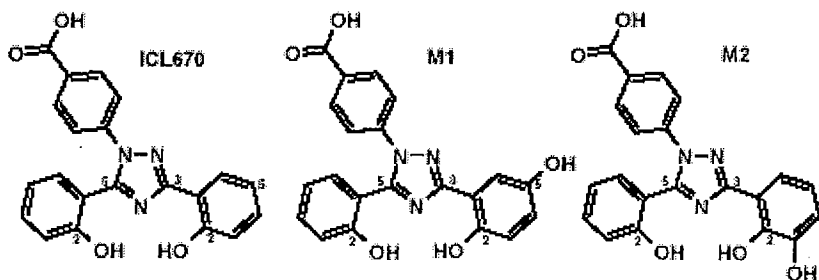
construct contains parts of the CYP3A4 promoter fused to a luciferase reporter gene. Additionally, the PXR gene was transfected into the cells in order to enhance the sensitivity of the assay system. The present study was conducted to evaluate the DPX2 reporter gene assay as a tool to assess PXR-mediated induction of CYP3A4. In this assay, cells were incubated with the test substances for 24 hours and the reporter gene expression (chemiluminescence) was measured using a kit []. In this assay, the sponsor used several positive and negative controls as mentioned in the results section.

Results: All tested substances with known PXR-mediated CYP3A4 induction potential (rifampicin, mevastatin, phenobarbital, mifepristone, corticosterone, clotrimazole, hydrocortisone, lovastatin and paclitaxel) were found to be positive in the DPX2 reporter gene assay. Some of the negative controls (ketoconazole, caffeine, and isoniazide) produced negative results as expected. However, several other negative controls such as 3-methylcholanthrene, β -naphthoflavone, clofibrate also weakly activated the reporter gene to a level corresponding to that of the positive control mevastatin. This indicated that that this assay might produces false positive results. Incubation with ICL670 led to a slight to moderate reporter gene activity at high concentrations (80-200 μ M).

2.6.4.6 Excretion

Excretion of Iron with Bile and Urine after Single Intravenous Administration of 50 mg/kg ICL670 and Two of its Metabolites (NVP-AHN496-NX-1 and NVP-AHK175-NX-1), respectively, to Rats (Study No. R0200705)

Methods: The aim of the study was to examine the excretion of iron in the bile and urine following intravenous administration of 50 mg/kg ICL670 or two of its metabolites NVP-AHN496-NX-1 (M1) and NVP-AHK175-NX-1 (M2), respectively, to bile duct-cannulated rats. Bile was collected at the following time points: 0-3, 3-6, 6-9, 9-12 and 2-24 h and urine was collected for 1-24 h. the samples were analyzed following []. The following figure (from page 4 of the study report) shows the structures of these metabolites.



Results: After intravenous administration of 50 mg/kg of ICL670 or its metabolites NVP-AHN496-NX-1 (M1) and NVP-AHK175-NX-1 (M2), the total mean excretion of Fe with bile and urine combined amounted to about 395 µg, 62 µg, and 70 µg within 0-24h post dose, respectively. Iron was predominantly excreted via bile. Excretion in the urine was about 10% of the amount eliminated in the bile. It is to be mentioned here that the normal iron excretion in rats (pre-dose) reported to be about 10 µg within 24 hours. It was concluded that the efficacy of ICL670 in its ability to transport Fe out of the body was found to be about 6-fold higher than that of its metabolites M1 and M2. No significant difference in the ability to eliminate Fe from the body was observed between the two metabolites.

2.6.4.7 Pharmacokinetic drug interactions

Inhibition of Human P450 Enzyme Activities by ICL670A and its Metabolite CGP82813A [Study No. DMPK(US) R99-07]

Methods: In this study, the inhibitory potential of ICL670 and its ferric complex form, CGP82813A, was investigated by assessing its capacity to inhibit specific P450 activities in human liver microsomes *in vitro*. The following reactions were studied: 1) inhibition of CYP1A2-dependent phenacetin O-deethylation, 2) inhibition of CYP2A6-dependent coumarin 7-hydroxylation, 3) inhibition of CYP2C8-dependent paclitaxel 6 α -hydroxylation, 4) inhibition of CYP2C9-dependent diclofenac 4'-hydroxylation, 5) inhibition of CYP2C19-dependent S-mephenytoin 4-hydroxylation, 6) inhibition of CYP2D6-dependent bufuralol 1'-hydroxylation, 7) inhibition of CYP2D6-dependent dextromethorphan O-demethylation, 8) inhibition of CYP2E1-dependent chlorzoxazone 6-hydroxylation and 9) inhibition of CYP3A4/5-dependent cyclosporine A metabolism. In all these reactions radioactive substrates were incubated with either ICL670 or CGP82813A (0-500 µM) for 5-30 minutes in the presence of NADPH (nicotinamide adenine dinucleotide phosphate) generating system (13.7 mM glucose 6-phosphate, 0.66 mM NADP⁺, and 2.8 U glucose 6-phosphate dehydrogenase) and the reactions were quenched by adding perchloric acid [E] and precipitated proteins were removed by centrifugation and resulting supernatant was analyzed by HPLC with radioactive detection.

Results: ICL670 inhibited paclitaxel 6 α -hydroxylation activity of CYP2C8 with an IC₅₀ value of 100 µM followed by CYP1A2 (phenacetin O-deethylation) with an IC₅₀ value of 175 µM. ICL670 also inhibited the activities of CYP3A4/5 (cyclosporine A metabolism), CYP2D6 (bufuralol 1'-hydroxylation), CYP2A6 (coumarin 7-hydroxylation), and CYP2C9 (diclofenac 4'-hydroxylation), with similar potencies (IC₅₀ values of 200, 200, 200, and 210 µM, respectively). CYP2C19 (S-mephenytoin 4-hydroxylation) activity was inhibited by ICL670 with an IC₅₀ value of 330 µM. However, CYP2E1 activity was inhibited at higher IC₅₀ (IC₅₀ > 500 µM). The ferric

complex of ICL670A, CGP82813A, attenuated phenacetin O-deethylation (CYP1A2) and coumarin 6-hydroxylation (CYP2A6) both with IC₅₀ values of 100 μM. All other CYP450 activities examined were inhibited by CGP82813A with IC₅₀ values ≥ 160 μM. The following table shows the IC₅₀ values of ICL670 and CGP82813A inhibition of specific human P450 activities (from page 26 of the study report).

Table 10: IC₅₀ values of ICL670A and CGP82813A inhibition of specific human P450 activities

Enzyme activity	Responsible human P-450	ICL670A IC ₅₀	CGP82813A IC ₅₀
phenacetin O-deethylation	CYP1A2	175 μM	100 μM
coumarin 7-hydroxylation	CYP2A6	200 μM	100 μM
paclitaxel 6α-hydroxylation	CYP2C8	100 μM	160 μM
diclofenac 4'-hydroxylation	CYP2C9	210 μM	n.d. ^a
S-mephenytoin 4-hydroxylation	CYP2C19	330 μM	250 μM
bufuralol 1'-hydroxylation	CYP2D6	280 μM	320 μM
dextromethorphan O-demethylation	CYP2D6	340 μM	> 500 μM
chlorzoxazone 8-hydroxylation	CYP2E1	> 500 μM	470 μM
cyclosporine A metabolism	CYP3A4/5	200 μM	> 500 μM

^a not determined

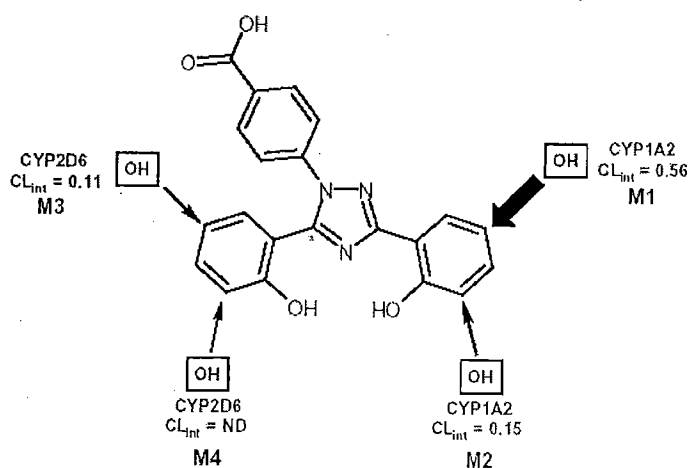
Overall, ICL670, inhibited cytochrome P450 (CYP) activities with IC₅₀ values ranging from 100 to > 500 μM. The ferric complex of ICL670, CGP82813, inhibited P450-dependent enzyme activities with IC₅₀ values ranging from 100 μM (CYP1A2, CYP2A6) to > 500 μM (CYP2E1, CYP3A4/5). It appeared that drug-drug interactions are possible for CYP2C8 substrates.

Identification of Human P450 Enzymes Metabolizing ICL670 and Determination of Potential Metabolic Interactions on its Metabolism [Study No. DMPK(US) R99-078]

Methods: The aim of the present study was to examine the potential metabolic pathways and kinetic parameters of ICL670 metabolism in human liver microsomes and to identify the human cytochrome P450 enzymes (CYP450) involved in the metabolism of ^{14}C -ICL670 (specific activity = $147 \mu\text{Ci}/\text{mg}$ or $5.44 \text{ MBq}/\text{mg}$) to assess the potential for drug-drug interactions. In addition, the structures of ICL670 metabolites formed by human cytochrome P450 enzymes were also identified by ^{13}C -

^1H and nuclear magnetic resonance (^{13}C -NMR). In this study, microsomal preparations from baculovirus-infected cells expressing human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, and CYP4A11 were used. Steady-state kinetic parameters of ICL670 metabolism were determined by incubation of human liver microsomes with 0-500 μM [^{14}C] ICL670 in the presence of a NADPH regenerating system, and incubated for 15 minutes at 37°C . Control incubations were performed by excluding the regenerating system or human liver microsomes. Reactions were quenched with trifluoroacetic acid (TFA): acetonitrile, and the precipitated protein were removed by centrifugation. The supernatants were analyzed by ^{13}C -HPLC with radioactive detection.

Results: The major metabolic pathways of ICL670 in human liver microsomes were the formation of four mono-hydroxylated metabolites, M1-M4. The enzymes involved in the metabolism were determined to be CYP1A1 (M1, M2), CYP1A2 (M1, M2) and CYP2D6 (M3, M4). The majority of the clearance in human liver microsomes was due to the formation of the mono-hydroxylated metabolite, M1, (CL_{int} , $0.16 \text{ ml} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$; V_{max} , $0.17 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$; K_m , $62 \mu\text{M}$) while the other products (M2-M4) contributed less to microsomal clearance (CL_{int} , $\sim 0.04 \text{ ml} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$; V_{max} , $0.47\text{-}0.54 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$; K_m , $700\text{-}840 \mu\text{M}$). CYP1A2 appeared to be the major enzyme for the oxidative metabolism of ICL670 in human liver (6-8-fold higher than 2D6). The following figure (from page 6 of the study report) shows the metabolic pathways of ICL670 in human liver microsomes.



Overall, the major metabolic pathways of ICL670 in human liver microsomes were the formation of four mono-hydroxylated metabolites, M1-M4. It is worth mentioning here that these four mono-hydroxylated metabolites were also found to be present in the bile and feces of rats and marmosets treated with a single dose of ICL670 in ADME studies as discussed before.

In vitro Analysis of Displacement of [¹⁴C] Warfarin or [¹⁴C] Diazepam from Human Plasma Proteins by ICL670 and its Iron Complex AML644 (Study No. R0400076)

Methods: The aim of this study was to identify and characterize possible interactions on the protein binding level between ICL670 and its iron complex AML644 with ¹⁴C-warfarin (6.68 MBq/mg) or ¹⁴C-diazepam (7.22 MBq/mg), typical ligands for the two main drug binding sites on human serum albumin. In previous study (R0201753), both, ICL670 and AML644, were found to be highly protein bound (> 99%). In this study, ICL670 and AML644 were tested at 268 and 134 μM maximum final concentrations, respectively. Plasma samples (n = 3) were spiked with the test articles and were incubated for 1 h at 37°C followed by ultracentrifugation. The total radioactivity in the ultrafiltrate was determined by LSC.

Results:

Displacement in human plasma

In human plasma, ICL670 displaced both [¹⁴C]diazepam (0.1 and 1 μg/mL) and [¹⁴C]warfarin (1 and 5 μg/mL). At a concentration of 100 μg/mL ICL670 (268 μM), the unbound fractions of [¹⁴C]diazepam and [¹⁴C]warfarin in human plasma were 3.4-3.6% and 1.5-1.6%, respectively, as compared to 2.3-2.4% and 1.1-1.2% in the absence of ICL670, respectively.

No increase in the unbound fraction of [¹⁴C]diazepam (0.1 and 1 μg/mL) and [¹⁴C]warfarin (1 and 5 μg/mL) occurred in human plasma at concentrations of up to 100 μg/mL of AML644 (134 μM).

Based on this displacement of both warfarin and diazepam, it appears that there is a potential for binding of ICL670 to albumin.

Evaluation of ICL670 as an Inhibitor of Acetaminophen Glucuronidation in Human Liver Microsomes (Study No. R0400198)

Methods: Many patients intended for treatment with ICL670 might also take acetaminophen as a concurrent medication. Since both drugs are metabolized mainly via glucuronidation, a drug-drug interaction of the two substances competing for UGTs might be expected. The present study was conducted to examine the potential inhibition of acetaminophen (0.25, 0.5, 1, 2, 3, 4, and 5 mM) glucuronidation by ICL670 (0, 50,

100, 200, and 300 μM) upon co-administration of both substances. Experiments were conducted *in vitro* using human liver microsomes. Microsomes were incubated with the test articles for 60 min and the reactions were stopped by adding perchloric acid and proteins were removed by centrifugation. *In vitro* formation of acetaminophen glucuronide was determined using a HPLC assay with UV detection.

Results: ICL670 displayed partial noncompetitive inhibition of acetaminophen glucuronidation in human liver microsomes with a K_i value of $204.8 \pm 28.7 \mu\text{M}$.

Interaction of ICL670 and its Possible Co-Medication Hydroxyurea with MDR1, MRP2 and MXR Efflux Transporters (Study No. R0400371)

Methods: The objective of this study was to identify the pump system (s) involved in ABC transporter-mediated drug efflux of ICL670 (which was previously identified as an ABC transporter substrate) and hydroxyurea (HU, a possible co-medication) and determination of the corresponding kinetic parameters (K_m , V_{max} and IC_{50}) (R0200436 and R0201161). This study was conducted by using ATPase and vesicular transport assay using membrane vesicles [(for MDR1 and MRP2) and from a selected mammalian cell line (for MXR). Briefly, efflux mediated by the human Multidrug-Resistant Protein 1 (MDR1), the human Multidrug-Resistant Associated Protein 2 (MRP2) or the human Breast Cancer Resistant Protein (MXR or BCRP) was measured by incubating the purified membrane vesicles in the absence and presence of stimulating agent (Verapamil, 40 μM for MDR1, Probenecid at 1mM for MRP2 and Sulfasalazine at 10 μM for MXR) with different concentrations of ICL670 and hydroxyurea (0.04, 0.13, 0.40, 1.21, 3.63, 10.89, 32.67 and 98.00 μM), respectively, in transport buffer at pH 7.4 at 37°C. The vesicular uptake assays were also conducted. Briefly, interaction of ICL670 or HU with the human MRP2 or MXR or BCRP was measured indirectly by incubating the purified membrane vesicles with known model substrates (LTC4 at 0.2 μM for MRP2 and E₁S at 0.5 μM for MXR) in the presence and absence of different concentrations of ICL670 and HU (0, 0.1, 1.0, 10.0 and 100.00 μM) in transport buffer at pH 7.4 at 23°C or 37°C, respectively.

Results: The results indicated an interaction of ICL670 with MXR ($K_m \cong 1 \mu\text{M}$) and MRP2 ($K_m > 10 \mu\text{M}$) efflux pumps suggesting ICL670 to be actively transported by these transporter proteins. In contrast, no activation of the MDR1 efflux protein could be observed. The results of the vesicular transport assays revealed that ICL670 can inhibit [³H]LTC4 as well as [³H]E1S transport mediated by MRP2 ($IC_{50} \cong 50 \mu\text{M}$) and MXR ($IC_{50} \cong 1 \mu\text{M}$), respectively. For hydroxyurea, up to a 100 μM concentration no activation and no inhibition of neither the MDR1, nor the MXR, nor the MRP2 proteins could be observed indicating HU not to be actively effluxed by any of these transporter systems.

Overall, it appeared that because of lack of overlapping substrate specificity, an interaction of ICL670 and HU on efflux transporter level (on MDR1, MRP2 and MXR) appeared unlikely.

Evaluation of Hydroxyurea as an Inhibitor of ICL670 *In Vitro* Metabolism in Human Liver Microsomes (Study No. R0400616)

Methods: Many patients suffering from β -thalassemia major or severe sickle cell anemia usually take high doses of hydroxyurea as a co-medication. The present study was conducted to examine the potential inhibitory effect of hydroxyurea (HU) on ICL670 metabolism. Experiments were conducted *in vitro* using human liver microsomes and ^{14}C -ICL670 (specific activity = 1.928 MBq/mg). In this study, pooled human liver microsomes were incubated with 50 μM ^{14}C -ICL670 in the presence of different concentrations of HU (0, 0.01, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5 mM) for 60 minutes at 37°C. The reactions were stopped by adding perchloric acid followed by centrifugation. The supernatants were analyzed by using HPLC with radiodetection.

Results: In the absence of HU, the HPLC chromatogram displayed the known *in vitro* metabolites of [^{14}C]ICL670: an acylglucuronide and two P450-dependent metabolites (as in study R0201162). Addition of hydroxyurea did neither change the metabolite pattern nor metabolism rates up to the highest concentration tested.

Overall, cytochrome P450- and UGT-mediated *in vitro* metabolism of ICL670 in human liver microsomes did not appear to be inhibited by hydroxyurea up to a concentration of 5 mM. Therefore, based on the results of this study, inhibition of ICL670 metabolism by hydroxyurea appears to be unlikely.

2.6.4.8 Other Pharmacokinetic Studies

The sponsor did not provide any other pharmacokinetic study report.

2.6.4.9 Discussion and Conclusions

Pharmacokinetics and disposition of ICL670, its metabolites, and the respective iron complex $\text{Fe-}[\text{ICL670}]_2$ were studied in mice, rats and marmosets and in part in dogs and rabbits. The fate of ICL670 and $\text{Fe-}[\text{ICL670}]_2$ appeared to be comparable across all species tested including man. The rat appeared to be the closest to human in terms of disposition and metabolism of ICL670. ICL670 crossed placental barrier in pregnant rats and rabbits. It appeared that ICL670 might have the potential to cross placental barrier in humans. ICL670 was also excreted into the milk of lactating rats; therefore, caution should be exercised when ICL670 is administered to a nursing women. ICL670 and/or its metabolites crossed the blood-brain barrier to a relatively low extent. Metabolism of ICL670 included mainly glucuronidation (animals and human), and to a lesser extent cytochrome P450-catalysed hydroxylation, mainly by CYP1A1, CYP1A2 and CYP2D6. Direct glucuronidation of ICL670 to the acyl-glucuronide (M3) occurred predominantly by UGT1A1 and UGT1A3. There appeared to be a possibility of the potential drug-drug interactions in terms of the cytochrome P450 (CYP1A, CYP2D6), glucuronosyl transferase enzymes, as well as hepatic anion transporters (MRP2, MXR). An interaction

potential of ICL670 and hydroxyurea in sickle cell anemia patients appeared to be unlikely.

2.6.4.10 Tables and figures to include comparative TK summary

The following table (from page 32 of the nonclinical summary overview) shows the exposure to ICL670 in animals at no-adverse-effect levels (NOAEL) compared to human exposure at the proposed dose of 20 mg/kg/day.

Table 5-8 ICL670 exposure multiples in toxicity studies

Species/ Study number	NOAEL ^b (mg/kg)	Sex	AUC _{0-24h} ^c ($\mu\text{mol}\cdot\text{h}/\text{L}$)	C _{max} ^c ($\mu\text{mol}/\text{L}$)	Exposure multiples	
					Based on AUC _{0-24h}	Based on C _{max}
4-wk rat [974080] ^d	10	male	25.8	6.43	0.02	0.05
	10	female	27.6	13.1	0.02	0.11
4-wk rat [987066] ^e	100	male	1095	276	0.78	2.3
	-	female	-	-	-	-
26-wk rat [987037] ^e	30 ^f	male	98	19.5	0.07	0.16
	30 ^f	female	166	72.3	0.12	0.60
4-wk marmoset [974194] ^g	65	male	520	92.7	0.37	0.77
	130	female	541	72.6	0.38	0.60
39-wk marmoset [982027] ^h	40	male	164	32.0	0.12	0.27
	40	female	195	37.0	0.14	0.31

^a based on 20 mg/kg, multiple dose, day 12, [study 0104], C_{max} = 120.5 $\mu\text{mol}/\text{L}$, AUC_{0-24h} = 1404 $\mu\text{mol}\cdot\text{h}/\text{L}$; ^b No-Observed-Adverse-Effect-Level; ^c based on day 1 data; ^d Standard diets; ^e Iron-supplemented diets; ^f Lowest dose in study, not NOAEL

The following table (from page 13 of the summary overview) shows the relevant pharmacokinetic parameters in rats, marmosets and humans.

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Table 4-1 Pharmacokinetic parameters of total ICL670

Single oral dose (mg/kg)	Human ~ 20 ^a	Marmoset 25	Rat ^{b, f} 10
AUC ($\mu\text{mol}\cdot\text{h}/\text{L}$)/(mg/kg) ^c	35.5	8.72 ^h	2.71 ^h
C _{max} ($\mu\text{mol}/\text{L}$)/(mg/kg) ^c	1.53	1.85	0.76
t _{max} (h)	4-6	0.5	0.5
Bioavailability (% of dose)	73 $\pm$ 20 ^d	88 ^b	32 ^b
ICL670 (% of ¹⁴ C-AUC)	91	25	67
t _{1/2} of ICL670 (h)	11 $\pm$ 5	0.7 (t _{1/2} 35) ^{e, f}	0.8
Reference	[study A0115]	[report R98-315]	[report R97-522]
Intravenous dose (mg/kg)	Human ^d 1.65	Marmoset 10	Rat ^{b, f} 10
AUC ($\mu\text{mol}\cdot\text{h}/\text{L}$)/(mg/kg) ^c	63.4	9.96 ^{b, k}	8.45 ^l
V _{ss} (L/kg)	0.18	8.1 ^{e, f}	0.64
Clearance CL (mL/min/kg)	0.74	3.2 ^f	5.6
Hepatic extraction ratio E _H (%) ^g	8	12	23
t _{1/2} of ICL670 (h)	4.1 $\pm$ 1.5	0.5 (t _{1/2} 51) ^{e, f}	0.7
Reference	[study A2101]	[report R98-315]	[report R97-522]

^a: an oral dose of 1000 mg ¹⁴C-labeled ICL670 was given as a drink suspension in water to thalassemia patients at steady-state (daily 1000 mg non-radiolabeled ICL670) [study A0115]

^b: calculations based on: $\text{ICL670}_{\text{total}} = \text{ICL670}_{\text{free}} + \text{Fe}-(\text{ICL670})_2$

^c: multiply $\mu\text{mol}/\text{L}$ or $\mu\text{mol}\cdot\text{h}/\text{L}$ with 373.37 to obtain $\mu\text{g}/\text{L}$ or $\mu\text{g}\cdot\text{h}/\text{L}$, respectively

^d: healthy volunteers, 130 mg, 90 min i.v. infusion versus 375 mg, p.o. [study A2101]

^e: value very high, probably due to substantial contribution by enterohepatic recirculation evident in the terminal elimination phase after 8 h

^f: parameter calculated by the author of this overview

^g: $E_H = \text{CL}/\text{hepatic plasma flow}$, where hepatic plasma flow = hepatic blood flow $\cdot$ hematocrit (HCT $\sim$ 0.45)

^h AUC_{0-72h}; ⁱ AUC_{0-24h}; ^j AUC_{0.083-24h}; ^k AUC_{0.083-72h}

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The pivotal absorption, distribution, metabolism and excretion (ADME) studies were previously listed under the section: **Studies reviewed within this submission.**

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The systemic toxicity of ICL670 was adequately evaluated in acute oral and intravenous toxicity studies in mice and rats, in subacute (4-Week) oral toxicity studies in mice, chronic (26-Week) oral (p.o.) and subacute (2-Week) intravenous (i.v.) toxicity studies in rats and chronic (39-Week) oral and subacute (2-Week) i.v. toxicity studies in marmoset monkeys. ICL670 was also tested in juvenile mice and rats to support its use in pediatric patients. The potential genotoxicity of ICL670 was examined in an adequate battery of genotoxicity tests. The carcinogenic potential of ICL670 has also been examined in p53

heterozygous mice (26-week) and Wistar Hannover rats (104-week). In addition, ICL670 has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats. Adequate safety pharmacology studies were also conducted with ICL670.

General Toxicology:

Acute toxicity studies were conducted in mice and rats after oral (50, 250, 500 and 1000 mg/kg in CD-1 mice; 50, 250, 500 and 1000 mg/kg in Wistar rats) and i.v. (5, 25, 50 and 150 mg/kg in CD-1 mice; 5, 25, 50 and 75 mg/kg in Wistar rats) administrations. In mice, the minimum lethal doses after oral and intravenous administration were 1000 and 150 mg/kg, respectively, in both sexes. In rats, the minimum lethal dose after oral administration was 500 mg/kg in both sexes. Following i.v. administration to rats, ICL670 was non-lethal up to 75 mg/kg in both sexes. Clinical signs in mice following oral or intravenous administrations included: discolored urine, lacrimation, ataxia, cold to touch, decreased defecation, decreased motor activity, dehydration, hunched posture, ptosis, distended abdomen, and labored breathing. Clinical signs in rats following oral or intravenous administration included decreased defecation and discolored urine.

Repeated dose toxicity studies were conducted in mice (up to 4 weeks at 10, 30, 100 and 200 mg/kg/day in iron-supplemented animals or at 100, 300 and 600→450 mg/kg/day in iron-supplemented animals and at 100 and 600 mg/kg/day in non-supplemented animals), rats (up to 26 weeks at 30, 80 and 180 mg/kg/day in iron-supplemented animals) and marmoset monkeys (up to 39 weeks at 20, 40 and 80 mg/kg/day in non-supplemented animals and at 80 mg/kg/day in iron-supplemented animals). The no-adverse-effect-levels (NOAEL) in mice, rats and marmoset monkeys were considered as 100 (Study No. 981060), 30 (Study No. 987037) and 40 mg/kg/day (Study No. 982027), respectively.

In mice, the target organs of toxicity appeared to be the kidney (tubular degeneration, tubular necrosis, regenerative changes, increased mitosis, basophilia, tubular vacuolation at 100 mg/kg/day in non-supplemented males and at 450 mg/kg/day in iron-supplemented animals), stomach (gastric erosions/ulcers, inflammation at 100 and 600 mg/kg/day in non-supplemented males and females, respectively, and at 450 mg/kg/day in iron-supplemented males and females), liver (necrosis and inflammation at 100 mg/kg/day in non-supplemented animals and at 450 mg/kg/day in iron-supplemented males), heart (multifocal myocytolysis in the ventricle at 600 mg/kg/day in both sexes with standard diet) and lymph node (lymphoid depletion at 100 mg/kg/day in non-supplemented mice and at 450 mg/kg/day in iron-supplemented males). In an oral toxicity study in juvenile mice at 5, 15 and 35 mg/kg/day in iron-supplemented animals and at 35 mg/kg/day in non-supplemented animals for up to 5 consecutive weeks from Day 7 *post partum*, the major target organ of toxicity appeared to be the thymus (lymphoid atrophy/necrosis at 35 mg/kg/day at Day 21 predominantly in males with or without iron supplementation). The juvenile mice (NOAEL = 5 mg/kg/day) appeared to be more sensitive to the toxicity of ICL670 when compared to adults. It is to be mentioned here that bilateral cataracts were observed at Week 15 at 200 and 300 mg/kg/day in iron-supplemented male and females, respectively, and in the 26-Week carcinogenicity study in p53 heterozygous

mice. Generally, non-iron-supplemented animals appeared to be more adversely affected compared to iron-supplemented animals. This could be partly due to higher drug exposure (AUC_{0-24h}) in animals with standard diet (males: 763 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 100 mg/kg/day at Day 1; Females: 618 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 100 mg/kg/day at Day 1) than the animals with iron-supplemented diet (Males: 297 $\mu\text{g}\cdot\text{hr}/\text{ml}$ $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 100 mg/kg/day at Day 1; Females: 180 $\mu\text{g}\cdot\text{hr}/\text{ml}$ $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 100 mg/kg/day at Day 1). Also, males appeared to be more sensitive to the toxicity of ICL670 than the females. This could be partly due to an apparent increase (almost 2-fold) in drug exposure in males ($AUC_{0-24h} = 2020$ $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 450 mg/kg/day at Week 4, iron-supplemented diet) when compared to females ($AUC_{0-24h} = 1170$ $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 450 mg/kg/day at Week 4, iron-supplemented diet).

In rats, the target organs of toxicity appeared to be the kidney (tubular vacuolation, necrosis in both sexes at 100 mg/kg/day with standard diet and iron-supplemented diet), gastrointestinal tract (stomach erosion and ulceration mainly in non-supplemented animals at 100 mg/kg/day), eye (bilateral cataract in 26-week study at 180 mg/kg/day starting at Week 11 and at all doses at Week 26), heart (myocardial degeneration predominantly in males at 100 mg/kg/day with standard diet), liver (single cell necrosis in males with standard diet at 100 mg/kg/day) and lymph node (lymphoid depletion, lymphoid hyperplasia at 100 mg/kg/day in animals with standard diet). ICL670 was also tested orally in juvenile rats (iron-supplemented) at 0, 10, 20 and 40→30 mg/kg/day from post partum day 7 through 70. The target organ of toxicity in juvenile rats appeared to be the eye (bilateral cataract at 20 and 40→30 mg/kg/day starting at Week 3) and kidney (inflammatory and degenerative changes consisting of tubular vacuolation and/or necrosis and tubular dilation and/or basophilia at 40→30 mg/kg/day in males and females). The NOAEL appeared to be 20 mg/kg/day in males and 10 mg/kg/day in females. Like juvenile mice, juvenile rats also appeared to be more sensitive to the toxicity of ICL670 when compared to adults. Generally, non-iron-supplemented animals appeared to be more adversely affected compared to iron-supplemented animals.

In marmoset monkeys, the target organs of toxicity appeared to be the kidney (tubular vacuolation, degeneration of renal cortical tubules at ≥ 80 mg/kg/day), gall bladder (inflammation, epithelial vacuolation, hyperplasia and fibrosis in males ≥ 85 mg/kg/day with standard diet), and liver (vacuolation of intrahepatic bile duct cells in males at 130 mg/kg/day with standard diet). Renal toxicities were generally less pronounced in iron-overloaded marmosets. It is to be noted here that in contrast to mice and rats, bilateral cataracts were not observed in any studies with the marmosets. It appears that there is a species difference in sensitivity to the ocular toxicity of ICL670. Generally, inflammatory and degenerative changes in the gall bladder and bile ducts were observed in the marmosets at highest tested doses (400 mg/kg/day ≤ 4 weeks and at 80 mg/kg/day in 39 weeks).

Genetic Toxicology: ICL670 was found to be negative in the Ames assay (Study 981683) and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes (Study 981803). In the *in vivo* rat (standard diet) bone marrow micronucleus test by oral route, ICL670 was found to be positive at 500 mg/kg dose level (Study 981866). In another oral bone marrow micronucleus test at

300 and 500 mg/kg/day (Study 981878, ± iron-loaded), ICL670 was negative. In a separate micronucleus study (982047 or Report No.1463/57-D5140) in rat liver hepatocytes at 125 and 250 mg/kg/day, ICL670 was negative.

Carcinogenicity: ICL670 has been tested in rats (104-Week) and p53 heterozygous mice (26-Week) following oral administration for its potential to cause carcinogenicity. ICL670 did not appear to show carcinogenicity in either rats or mice at the tested doses.

Reproductive Toxicology: Reproduction studies have been performed in pregnant rats at oral doses up to 100 mg/kg/day (about 0.8 times the recommended human oral dose based on the body surface area) and in pregnant rabbits at oral doses up to 50 mg/kg/day (about 0.8 times the recommended human oral dose based on the body surface area) and have revealed no adverse effects on fertility or harm to the fetus due to ICL670. In pre- and post-natal developmental study in rats up to 90 mg/kg/day dose (0.7 times the recommended human oral dose based on the body surface area), ICL670 caused an increase in the number of stillbirths at 90 mg/kg/day. ICL670 also caused an increased incidence of renal anomalies (short/absent renal papilla, fluid-filled kidney) in F1 pups at 30 mg/kg/day (about 0.2 times the recommended human oral dose based on the body surface area). However, these effects were not considered as teratogenic effects as kidney toxicities were also found in the neonatal rats and were considered as the nephrotoxicity associated with the ICL670.

Special Toxicology: In rats, ICL670 showed effects on immune parameters, which included a dose-related decrease in white blood cells and lower IgG titers, and a decrease in absolute peripheral and tissue B cells in males at 60 mg/kg/day. Based on the results of the mechanistic studies (in cell cultures of porcine or human kidney proximal tubule cells and in rat kidney slices) regarding the nephrotoxicity of ICL670, it appeared that ICL670-induced nephrotoxicity may be partly attributable to iron depletion. Results of mechanistic studies using rat lenses, it appeared that oxidative stress might be partly involved in ICL670-induced cataract.

Toxicological qualification of []:

[] is an impurity of ICL670, which was carried over from the synthesis of ICL670 and was not considered as a degradation product formed during processing or storage. The structure of [] is shown below.

[]

1

As [] are generally considered as genotoxic substances, the sponsor conducted Ames assay (Study No. 0212003) and chromosome aberration assay using human

peripheral blood lymphocytes (Study No. 0212201). [] was found to be positive in Ames assay in TA97A (frameshift mutation) and negative in chromosome aberration assay. Toxicological qualification of this impurity present in the Drug Substance at a maximum level of μg (parts per million) is important due to its genotoxic and carcinogenic potential. [] has been reported to be weakly positive in *Salmonella typhimurium* strain TA104 at 50 μg /plate concentration []

[] In addition, [] has also been reported to be a potential carcinogen [] Several batches of ICL670 containing this impurity have been tested in different toxicology studies. Based on sponsor's maximum limit of μg [] of [] in the drug substance []

[]), the maximum clinical dose (MCD) of [] would be [] mg/kg (base on a 50 kg body weight and a dose of 20 mg/kg/day). The calculated NOAEL (from the NOAEL of ICL670) for [] in the 26-week oral study (Study No. 987037, Batch 97902, [] ppm or μg of [] per kg of ICL670 as per above table) in rats (NOAEL for ICL670 = μg / kg or μg / kg) is [] (please see page 263 for details) mg/kg, which offers a safety margin of [] The NOAEL for [] in the 39-week oral study (Study No. 982027, Batch 98903, [] ppm or μg of [] per kg of ICL670 as per above table) in marmoset monkeys (NOAEL for ICL670 μg / kg or μg / kg) is [] (please see page 263 for details) mg/kg, which allows a maximum dose [] of μg / kg or [] mg/day (μg / ppm) based on a 5-fold margin of safety. Therefore, sponsor's maximum limit of μg / ppm is about 12.5 times more than the maximum allowable amount of μg / ppm based on toxicology study. The sponsor may be asked to reduce the level of [] to μg / ppm or conduct a 2-week oral toxicity study with [] in rats to qualify this impurity in the drug substance at the proposed maximum limit of μg / ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

2.6.6.2 Single-dose toxicity

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TOXICOLOGY:**Acute Toxicity in Mouse and Rat**

Report No.	Testing Laboratory	Species & Route	Date Started	Date Completed	Batch No.
987027	Novartis Pharmaceuticals Corp., East Hanover, NJ, USA	CD-1 (ICR) BR Mice, Oral (gavage)	3/23/1998	4/10/1998	97902
987026	Same as above	Same as above, IV	3/23/1998	4/13/1998	97902
987010	Same as above	HsdBrl:W H (Wistar Hannover Rats), Oral (gavage)	4/13/1998	4/27/1998	97902
987009	Same as above	Same as above, IV	4/13/1998	4/27/1998	97902

GLP Compliance: Statements of compliance with GLP regulations and the quality assurance unit were included.

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Methods: The acute toxicity of ICL 670 was examined in mice and rats. Animals were observed daily for 15 days post dose. For mice receiving drugs at doses of 50, 250, 500 mg/kg by oral gavage (ICL 670 in 0.5% Klucel), the dose volume was 20 ml/kg. For mice that received drug by intravenous route [(ICL 670 in PEG 400, NF/purified water, USP (60:40)] at 5 (0.5 mg/ml), 25 (2.5 mg/ml), 50 (5.0 mg/ml), and 150 (15 mg/ml) mg/kg, the dose volume was 10 ml/kg. Wistar rats received the drug at 50, 250, 500, and 1000 mg/kg by oral route; the dose volume was 10 ml/kg. For Wistar rats that received ICL 670 at 5, 25, 50, and 75 mg/kg by intravenous route, the dose volume was 5 ml/kg.

Results: For CD-1 mice administered ICL 670 by oral gavage, the minimum lethal dose in both males and females was 1000 mg/kg. The maximum non-lethal dose in both sexes was 500 mg/kg when given by oral gavage in mice. The no observed effect level (NOEL) was considered to be 50 mg/kg when given by oral gavage in mice. For CD-1 mice administered ICL 670 by intravenous route, the minimum lethal dose was found to be 150 mg/kg and the maximum non-lethal dose was found to be 50 mg/kg. Clinical signs in mice following oral or intravenous administration included: 1) minimal signs of discolored urine and lacrimation (at 250 and 500 mg/kg); 2) ataxia, cool to touch, decreased defecation, decreased motor activity, dehydration, hunched posture, lacrimation, ptosis in both sexes, distended abdomen, labored breathing in males at 1000 mg/kg; 3) decreased body weight and food consumption at 500 mg/kg in both sexes. All animals appeared asymptomatic by Day 2 and all surviving males at 1000 mg/kg recovered by Day 4.

For rats administered ICL 670 by oral gavage, the minimum lethal dose was 500 mg/kg in both the sexes. The maximum non-lethal dose was 250 and 500 mg/kg in females and males, respectively. The NOEL for ICL 670 when given by oral route was 250 mg/kg. When given by intravenous route, there was no mortality at any of the tested doses. The maximum non-lethal dose was 75 mg/kg in both the sexes and the NOEL was 50 mg/kg. Clinical signs in rats following oral or intravenous administration included decreased defecation and discolored urine in males and decreased defecation in females at 1000 mg/kg (oral) and discolored urine in both sexes at 75 mg/kg (intravenous).

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Acute Toxicity Studies With ICL 670 in Mice and Rats

Species	Route	Dose (mg/kg)	Maximum Nonlethal Dose (mg/kg)		Minimum Lethal Dose (mg/kg)		Time to death
			Male	Female	Male	Female	
CD-1 Mice, 3-5/sex/ Dose	Oral	50	500	500	1000	1000	Day 2-5
		250					
		500					
		1000					
CD-1 Mice, 5/sex/ Dose	IV	5	50	50	150	150	Day 3
		25					
		50					
		150					
Wistar Rats, 3/sex/ Dose	Oral	50	500	250	500	500	Day 4 (500 mg/kg), Day 2 & 9 (1000 mg/kg)
		250					
		500					
		1000					
Wistar Rats, 3/sex/ dose	IV	5	75	75	No death	No death	None
		25					
		50					
		75					

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For CD-1 mice administered ICL 670 by oral gavage, the minimum lethal dose in both males and females was 1000 mg/kg. The maximum non-lethal dose in both sexes was 500 mg/kg when given by oral gavage in mice. The no observed effect level (NOEL) was considered to be 50 mg/kg when given by oral gavage in mice. For CD-1 mice administered ICL 670 by intravenous route, the minimum lethal dose was found to be 150 mg/kg and the maximum non-lethal dose was found to be 50 mg/kg. The LD-50 was not determined in mice when given by intravenous route. For rats administered ICL 670 by oral gavage, the minimum lethal dose was 500 mg/kg in both the sexes. The maximum non-lethal dose was 250 and 500 mg/kg in females and males, respectively. The LD-50 in rats was not determined. The NOEL for ICL 670 when given by oral route was 250 mg/kg. When given by intravenous route, there was no mortality at any of the tested doses. The maximum non-lethal dose was 75 mg/kg in both the sexes and the NOEL was 50 mg/kg.

2.6.6.3 Repeat-dose toxicity

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Subacute Toxicity

Mouse

Comparative 4-Week Oral Toxicity Study in Mice (Report 95-6158).

Testing Laboratory: E

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Date Started: January 15, 1996

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Date Completed: February 19, 1996

GLP Compliance: Statements of compliance with GLP provided but states that "there was no independent monitoring of the various phases of the study and the report by the Quality Assurance Unit, and the final report of the study does not contain analytical and/or stability data pertaining to the test article" (from vol. 7, page 8-726 of sponsor's submission). Several investigations were performed by a technician from the Research Department who does not work under the formal standards of GLP.

Animals: Albino female Mice, Tif:MAGf (SPF) were used throughout the study. Animals were 5-10 weeks of old at the time of dosing and body weight range was 24.3-32.3 g.

Drug Batch: ICL 670, Batch number 4.

Methods: This study was performed to obtain decision-relevant information on the oral toxicity of 9 different new classes of potential iron chelators including ICL 670 in mice. However, this review was focused on the studies with ICL 670 only as the IND 58, 544 were submitted for this drug. The studies with other drugs were not reviewed for the purpose of this submission. Female mice (5/dose) were administered orally with ICL 670 at doses of 134 and 268 mg/kg once daily for 5 days a week for the first 3 weeks, and 7 days in the last week. The dose volume was 10 ml/kg. The dose selection was made based on the prior knowledge of toxicological/pharmacological properties of the substance (details of the studies were not mentioned). Control animals received Klucel HF (Hydroxypropylcellulose 0.5%). Animals were observed for clinical signs daily during drug administration, 3 times a week during pretest. Body weight was taken daily during drug administration and 3 times a week during pretest. Hematology and clinical chemistry were performed terminally. The following organs were weighed at necropsy: kidneys, liver, spleen and thymus. Gross and histopathology studies were performed on selected organs: kidneys, liver, small intestine, spleen, sternum, and thymus.

Results:

1. **Clinical Signs:** One (1/5) animal showed decreased activity, stiff gait, hunched posture, sunken flanks and piloerection (slight to moderate) at the end of the treatment.
2. **Mortality:** None.
3. **Body Weight:** The initial and final body weights of the control females were 25.2 and 28.9 g, respectively. No treatment-related changes were observed.
4. **Hematology:** There was an increase in RBC parameters [(7.4% increase in hemoglobin (Hb) compared to control (9.5 nmol/L); 11% increase in RBC compared to control (9.61 T/L); 4% increase in hematocrit (Hct) compared to control (0.47)] at 268 mg/kg dose level.

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5. **Blood Chemistry:** Cholinesterase activity (AChE) was increased by 9.5% compared to control (4832 U/L) at 268 mg/kg. About 3 and 12% decrease in urea at 134 and 268 mg/kg dose level value, respectively compared to control (8.58 mmol/L). About 35% and 8% increase in creatinine value was observed at 134 and 268 mg/kg dose levels, respectively compared to control (17.1 μ mol/L). About 8 and 11% increase in protein values was reported at 134 and 268 mg/kg dose levels, respectively compared to control (49.9 g/L).
6. **Organ Weights:** Thymus weight was decreased significantly by 37% at 268 mg/kg dose level compared to control (0.060 g). Kidney weight was increased by 14% at high dose group compared to control (0.368 g).
7. **Histopathology:** Thymus atrophy was observed at both the dose levels in all animals 5/5). Tubular degeneration in the kidneys was observed in 4/5 animals at 268 mg/kg doses. In the liver, centrilobular hypertrophy and microvacuolation were observed at 268 mg/kg dose in 3/5 animals. Atrophy of the spleen was observed in 1/5 animals at high dose level.

In 4-week toxicity study in mice, animals were treated with 134 and 268 mg/kg doses orally for 4 weeks. NOEL was not established; however, 134 mg/kg may be considered as a tolerated dose. Target organ of toxicity appeared to be kidney (tubular degeneration at 268 mg/kg), thymus (atrophy at both dose levels), liver (centrilobular hypertrophy and microvacuolation at 268 mg/kg) and spleen (atrophy of the spleen at 268 mg/kg dose level).

4-Week Oral Dose-Range Finding Study in Mice with Iron-Supplemented Diet (Study No. 981060)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: November 3, 1998

Date Completed: December 2, 1998

GLP Compliance: A statement of compliance was included.

Animals: C57BL/6TacBR N5 wild type mice were used throughout the study. Animals were 8-9 weeks old at the initiation of dosing and body weight ranges from 18-31 g at the start of the treatment.

Drug Batch: ICL 670. Batch No. 97902.

Methods: This study was conducted to aid in the selection of appropriate doses for a subsequent 26-week carcinogenicity study in transgenic mice. Mice (8/sex/group) were administered orally (gavage, dose volume = 10 ml/kg) with ICL 670 at 10, 30, 100, and 200 mg/kg/day (test article concentration was 0, 1, 3, 10, and 20 mg/ml), once a day (7 days a week) for 4 weeks. Control animals received 0.5% Klucel at the same dose volume. The animals were given iron-supplemented diet (at a concentration of 1680 mg/kg) throughout the study. Animals were observed daily for clinical signs and mortality. Body weight was recorded daily throughout the treatment period. Food consumption was recorded once a week during the treatment period.

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Hematology and serum chemistry was performed on day 29 (males) and day 30 (females). All animals were sacrificed at the end of the treatment period and subjected to necropsy. Absolute and relative weights were determined for brain, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, and uterus. Histopathological examinations were performed on the following tissues: adrenals, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerves, gall bladder, Harderian glands, heart, ileum, jejunum, kidneys, knee joint, lacrimal glands, liver, lungs, lymph node (mandibular, bronchial, and mesenteric), mammary gland, ovaries, pancreas, peripheral nerves, pituitary, prostate, rectum, salivary gland, seminal vesicles, skeletal muscles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus, and vagina.

Results:

1. **Observed Effects:** No treatment-related signs were observed.
2. **Mortality:** None.
3. **Body Weight:** The mean initial and final body weights of the control males were 27.4 and 29.7 g, respectively. The mean initial and final body weights of the control females were 19.6 and 22.3 g, respectively. The body weights were significantly reduced at 10 (94% of control), 30 (91% of control), and 100 (88% of control) mg/kg/day dose levels in the males at week 4. However, the body weights were not significantly altered in the females at this time point compared to control.
4. **Food Consumption:** The mean initial and final food consumption in control male were 5.5 and 4.4 g/animal/day, respectively. The mean initial and final food consumption in control females were 4.3 and 3.8 g/animal/day, respectively. The food consumption was not affected by treatment.
5. **Hematology:** There were no treatment-related changes.
6. **Clinical Chemistry:** There were no treatment-related changes.
7. **Organ Weights:** Thymus (70% and 73% of control in males and females, respectively, control male = 0.053 g and control female = 0.067 g), spleen (84% of control in females, control female = 0.088 g), liver (88% of control in males, control male = 1.46 g) weights were altered at 200 mg/kg/day dose level.
8. **Gross Pathology:** No treatment-related changes were observed.
9. **Histopathology:** Histopathological changes were observed in the epididymis (granulomatous, ductal dilatation, sperm stasia, oligospermia at 200 mg/kg dose), liver (decreased glycogen content in males at 200 mg/kg) and stomach (focal alteration in the glandular stomach characterized by inflammation, hypertrophy of the chief cells, atrophy of the parietal cells at all dose levels in both the sexes).

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10. **Toxicokinetics:** The pharmacokinetic parameters were determined from a 4-week oral dose-range finding study in mice. Mice (8/sex/group) were administered with 10, 30, 100 or 200 mg/kg/day of ICL 670 for at least 27 days orally. The toxicokinetic parameters are summarized below (from volume 18, page 8-4546 of sponsor's submission):

Time	Dose (mg/kg)	Male Mice			Female Mice		
		C _{max}	T _{max}	AUC	C _{max}	T _{max}	AUC
Day 2	10	1.52	0.5	3.77	3.15	0.5	4.92
	30	10.9	0.5	18.9	21.9	0.5	39.5
	100	54.0	2	217	62.8	0.5	213
	200	173	2	1040	132	2	746
Week 4	10	1.5	0.5	4.35	4.4	0.5	7.54
	30	11.9	0.5	24.5	10.2	0.5	21.3
	100	70.6	0.5	205	42.8	0.5	148
	200	140	2	660	123	2	656

C_{max} (µg/ml); T_{max} (hr); AUC (0-24h) (µg.h/ml).

ICL 670 was found to be rapidly absorbed in mice and the absorption appeared to be delayed with increasing doses. The systemic exposure of mice to total ICL 670 was increased overproportionately with doses based on the AUC values. No systemic sex-related difference in AUC values was observed.

In a 4-week oral dose range-finding study in mice, animals were administered with 10, 30, 100 or 200 mg/kg/day of ICL 670 orally (gavage). The no-toxic-effect-level was established as 100 mg/kg/day; however, 200 mg/kg/day could be considered as a tolerated dose. The target organ of toxicity appeared to be epididymis (granulomatous, ductal dilatation, sperm stasia, oligospermia at 200 mg/kg dose), liver (decreased glycogen content at 200 mg/kg) and stomach (focal alteration in the glandular stomach characterized by inflammation, hypertrophy of the chief cells, atrophy of the parietal cells at all dose levels).

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TOXICOLOGY**Study title: 4-Week Oral (Gavage) Dose Ranging Study in C57BL/6 Mice**

Key study findings: The MTD in iron-supplemented males and females appeared to be 200 and 300 mg/kg/day, respectively. In case of animals with standard diet, the MTD in males appeared to be <100 mg/kg/day based on the observed mortality; however, in females with standard diet, the MTD appeared to be 100 mg/kg/day based on observed mortality at 600 mg/kg/day and lack of histopathological findings at 100 mg/kg/day.

Report no./Study no: Study No. 0170121

Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Date of study initiation: November 27, 2001

Date of study completion: September 25, 2002

GLP compliance: A statement of compliance was included.

QA report: yes (x) no ()

Drug, lot #, and % purity: ICL-670, Batch No. 9923008, []

Formulation/vehicle: Suspension/0.5% (w/v) Klucel, NF in water.

Methods: The purpose of this study was to establish the toxicity of ICL-670 in iron-supplemented (approximately 1680 ppm $\pm$ 10%) and non-iron-supplemented C57BL/6 mice (p53 wild-type). In this study, iron-supplemented animals (10/sex/dose) were treated with ICL-670 by oral gavage as a suspension in aqueous 0.5% (w/v) Klucel at 100, 300 and 600 $\rightarrow$ 450 mg/kg/day (10 ml/kg). Due to excessive mortality/moribundity at 600 mg/kg/day following two doses, dosing was suspended on Days 3 – 7 and resumed on Day 8 at 450 mg/kg/day. Two additional groups of mice (10/sex/group) received 0.5% (w/v) Klucel (10 ml/kg) and served as controls (Group 1 = standard diet and Group 2 = iron-supplemented diet). The animals (10/sex/group) with standard diet were treated at 100 and 600 mg/kg/day. The dose selection was based on the results of the previous 4-week dose ranging study (study no. 981060) in iron-supplemented C57BL/6 (wild-type) mice at 10, 30, 100 and 200 mg/kg/day.

Dosing:

Species/strain: Mice/C57BL/6 (wild-type)

#/sex/group or time point (main study): 10/sex/group

Satellite groups used for toxicokinetics or recovery: 8/sex/group

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Age: 8 – 11 weeks

Weight: Male: 24.3 – 30.3 g; Female: 20.3 – 26.1 g

Doses in administered units: Iron-supplemented diet: 100, 300 and 600 → 450 mg/kg/day
Standard diet: 100 and 600 mg/kg/day

The following table (from 17 of sponsor's submission) shows the study design.

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Table 3-2 Study design, animal allocation and test article doses

Group	Number/sex	Animal Numbers		Dose (mg/kg/day)	Concentration (mg/mL)
		males	females		
1 Control (standard feed)	10 main study	1001-10	1501-10	0	0
	2 toxicokinetic	1011-12	1511-12		
2 Control (feed with iron)	10 main study	2001-10	2501-10	0	0
	2 toxicokinetic	2011-12	2511-12		
3 Low (feed with iron)	10 main study	3001-10	3501-10	100	10
	8 toxicokinetic	3011-18	3511-18		
4 Mid (feed with iron)	10 main study	4001-10	4501-10	300	30
	8 toxicokinetic	4011-18	4511-18		
5 High (feed with iron)	10 main study	5001-10	5501-10	600→450*	60→45
	8 toxicokinetic	5011-18	5511-18		
6 Low (standard feed)	10 main study	6001-10	6501-10	100	10
	8 toxicokinetic	6011-18	6511-18		
7 High (standard feed)	10 main study	7001-10	7501-10	600**	60
	8 toxicokinetic	7011-18	7511-18		

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Animals were acclimated to fasting procedures approximately 1 week prior to the start of dosing (approximately 6 hours each day).

*Dosing at 600 mg/kg/day was suspended on days 3-7 and resumed at 450 mg/kg/day on day 8.

**Dosing at 600 mg/kg/day was suspended on day 3 and all animals were removed from the study by day 4.

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Basis of dose selection: The dose selection was based on the results of the previous 4-week dose ranging study (study no. 981060) submitted under Amendment # 017 dated July 6,

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2001 in iron-supplemented C57BL/6 mice at 10, 30, 100 and 200 mg/kg/day. This study was previously reviewed (pharmacology review dated July 31, 2001) and presented at the Exec CAC held on August 7, 2001.

Route, form, and volume: Oral, suspension, and 10 ml/kg

Observations and times:

Clinical signs: Clinical signs were observed on a daily basis.

Body weights: Body weights were recorded on Day 1, 8, 14, 21 and 29.

Food consumption: Food consumption was not measured due to excessive feed spillage.

Hematology: Blood samples were collected for hematology at necropsy.

Clinical chemistry: Blood samples were collected for serum chemistry at necropsy.

Ophthalmoscopy: Ophthalmoscopic examinations were performed at pretest and during Week 4 from all surviving animals from dose Groups 1 – 6.

Gross pathology: Gross pathology was conducted at necropsy.

Organs weighed: The following organs were weighed from all main study animals: brain, ovaries, spleen, heart, kidney, liver, testes, and thymus.

Histopathology: The following organs/tissues were examined for histopathology from all main study animals: adrenals, aorta, bone marrow, brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eye, femur/tibia, gall bladder, ovaries, parathyroid, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscles, skin, spinal cord, spleen, Harderian gland, heart, ileum, jejunum, kidney, lacrimal gland, liver, lung, lymph node (bronchial, mandibular, mesenteric), mammary gland, sternum, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Toxicokinetics: Satellite animals were bled for toxicokinetics after the first dose and at the end of the dosing phase. For control Groups 1 and 2, 2 animals/sex were bled at approximately 0.5 hours post-dose. For ICL-670 treated animals, 2/sex/timepoint were bled at approximately 0.5, 2, 6 and 24 hours post-dose for both iron-supplemented and standard diet. The samples were analyzed by high pressure liquid chromatography (HPLC).

Tissue iron analysis: Iron content in the liver and kidney was measured. However, the details of the methods were not mentioned.

Results:

Mortality:

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Iron-Supplemented Diet:

There was no mortality at 100 or 300 mg/kg/day. Test article-related mortality was seen in both sexes at 600→450 mg/kg/day. In males, three animals were found dead on Day 4 following two doses at 600 mg/kg/day. After the dose was reduced to 450 mg/kg/day (on Day 8), two males died on Days 12 and 25, respectively, and one male was sacrificed moribund on day 26. In females, at 600→450 mg/kg/day, one animal died on day 27. The causes of deaths were not discussed.

Standard Diet:

There was no mortality in females at 100 mg/kg/day; however, six males died between Days 13 and 25 and one male was sacrificed in moribund condition on Day 14. At 600 mg/kg/day, all of the animals died or were sacrificed in moribund on Days 3 or 4, following 2 daily doses of ICL-670. The causes of deaths were not mentioned.

Clinical signs: No test-article-related clinical signs were observed at 100 or 300 mg/kg/day. Test article-related signs in iron-supplemented animals at high dose included reduced feces, cold to touch, hunched posture, decreased locomotor activity, lacrimation, ptosis, ataxia, impaired righting reflex, piloerection, red urine, wet perineal staining and/or irregular respiration. In non-iron-supplemented animals, above-mentioned drug-related clinical signs were also observed mainly in males at both 100 and 600 mg/kg/day doses.

Body weights:

Iron-Supplemented Diet:

The mean initial and final weight of control males was 27.1 and 30.9 g, respectively. The mean initial and final weight of control females was 22.6 and 24.8 g, respectively. Final body weights in males were 98, 94 and 88 % of control at 100, 300 and 600→450 mg/kg/day, respectively. In males, the body weight gain was suppressed by 8%, 18% and 84% at 100, 300 and 600→450 mg/kg/day, respectively, compared to control weight gain. In contrast, the body weight gain in females was increased by 9%, 23% and 64% at 100, 300 and 600→450 mg/kg/day, respectively, when compared to control weight gain.

Body Weight (g) of Males

Treatment (mg/kg/day)	Initial	Day 29	Weight Gain (g)	Suppression of Weight Gain (% Control)
0	27.1	30.9	3.8	0
100	26.8	30.3	3.5	7.9
300	26.1	29.2	3.1	18.4
600/450	26.7	27.3	0.6	84.2

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Body Weight (g) of Females

Treatment (mg/kg/day)	Initial	Day 29	Weight Gain (g)	Weight gain (% of Control)
0	22.6	24.8	2.2	100.0
100	22.7	25.1	2.4	109.0
300	22.7	25.4	2.7	122.7
600/450	21.7	25.3	3.6	163.6

Standard Diet:

The mean initial and final body weight of control males was 26.8 and 30.4 g, respectively. The mean initial and final body weight of control females was 22.1 and 24.6 g, respectively. The body weight gain was suppressed by 92% in males at 100 mg/kg/day compared to control. In contrast, in females, body weight gain at 100 mg/kg/day was similar to control weight gain.

Body Weight (g) of Males

Treatment (mg/kg/day)	Initial	Day 29	Weight Gain (g)	Suppression of Weight Gain (% Control)
0	26.8	30.4	3.6	0
100	26.3	26.6	0.3	91.7
600	26.7	-	-	-

Body Weight (g) of Females

Treatment (mg/kg/day)	Initial	Day 29	Weight Gain (g)	Weight gain (% of Control)
0	22.1	24.6	2.5	100.0
100	22.4	24.9	2.5	100.0
600	22.7	-	-	-

Hematology: No significant treatment-related changes were observed at 100 mg/kg/day in either sex in animals on iron-supplemented diet.

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Iron-Supplemented Diet:

The following treatment-related increases were observed: erythrocytes (male and female: 113% of control at 300 mg/kg/day, control = 10.05 and 10.14 M/ μ l in males and females, respectively), hemoglobin (male: 116% of control at high dose; female: 115% of control at high dose; control: 15.2 and 15.4 g/dl in males and females, respectively), hematocrit (male: 114% of control at 300 mg/kg/day, control = 46.4%; female: 117% of control at high dose, control = 46.7%), mean corpuscular volume (female: 104% of control at high dose, control = 46.1 fl) and reticulocyte (female: 204% of control at high dose, control = 2.4%).

Standard Diet:

The following treatment-related increases were observed at 100 mg/kg/day: erythrocytes (male: 116.5% of control, control = 10.21M/ μ l; females: 104% of control, control = 9.97 M/ μ l), hemoglobin (male: 113% of control, control = 15.1 g/dl), hematocrit (male: 112% of control, control = 46.2%; female: 107% of control, control = 45.0%), mean corpuscular volume (male: 95.6% of control, control = 45.3 fl; female: 103% of control, control = 46.2 fl) and reticulocyte (male: 206% of control, control = 3.5%).

Clinical chemistry:*Iron-Supplemented Diet:*

In males, serum alanine amino transferase (ALT, 367% of control, control = 36 U/l), total protein (116% of control, control = 5.5 g/dl), albumin (116% of control, control = 3.7 g/dl), globulin (117% of control, control = 1.8 g/dl) values were increased at 600 mg/kg/day compared to control. In addition, calcium (110% of control at high dose, control = 9.4 mg/dl), cholesterol (134% and 193% of control at 100 and 600 mg/kg/day, respectively, control = 100 mg/dl) and triglyceride (137% of control at 100 mg/kg/day, control = 67 mg/dl) concentrations were increased in males at $\geq$ 300 mg/kg/day. In females, calcium (104% of control at high dose, control = 9.7 mg/dl), phosphorus (112% of control, control = 7.2 mg/dl), and cholesterol (123% and 148% of control at 100 and 600 mg/kg/day, respectively, control = 75 mg/dl) concentrations were increased at 600 $\rightarrow$ 450 mg/kg/day compared to control.

Standard Diet:

In males, serum alanine amino transferase (494% of control, control = 35 U/l), aspartate amino transferase (AST, 184% of control, control = 126 U/l), alkaline phosphatase (ALP, 382% of control, control = 86 U/l), total protein (109% of control, control = 5.6 g/dl), albumin (105% of control, control = 3.8 g/dl), globulin (117% of control, control = 1.8 g/dl) values were increased at 100 mg/kg/day compared to control. In addition, phosphorus (129% of control, control = 7.2 mg/dl), cholesterol (170% of control, control = 104 mg/dl) and triglyceride (151% of control, control = 77 mg/dl) concentrations were also increased in males at 100 mg/kg/day compared to control. In females, only calcium (104% of control, control = 9.4 mg/dl) and triglyceride (121% of control, control = 53 mg/dl) concentrations were increased at 100 mg/kg/day.

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Ophthalmoscopy: There were no treatment-related findings.

Organ weights: In iron-supplemented mice, weights of kidney (male: 108% of control, control = 0.399 g; female: 112% of control, control = 0.310 g), liver (male: 123% of control, control = 1.230 g; female: 117% of control, control = 1.00 g) and spleen (male: 150% of control, control = 0.056 g; female: 124% of control, control = 0.068 g) were increased at 600→450 mg/kg/day in both sexes. Thymus weights were decreased in males at 300 mg/kg/day (61% of control, control = 0.036 g) and in both sexes at 600→450 mg/kg/day (male: 56% of control, control = 0.036 g; female: 76% of control, control = 0.046 g). In animals fed with standard diet, liver weights were increased in both sexes at 100 mg/kg/day (male: 126% of control, control = 1.152 g; female: 115% of control, control = 0.941 g) whereas thymus weights were decreased in both sexes (male: 33% of control, control = 0.039 g; female: 90% of control, control = 0.048 g).

Gross pathology: The following changes were observed: enlarged spleen in 3 males and 2 females at 600→450 mg/kg/day (iron-supplemented), small thymus at 600→450 mg/kg/day (iron-supplemented) in 4 males at 100 mg/kg/day (standard diet). In addition, many treated mice had dark contents in their ileum, jejunum, cecum and/or colon.

Histopathology:

Iron-Supplemented Diet:

Histopathological changes were observed at 600→450 mg/kg/day in the stomach (gastric erosions and/or ulcers and inflammation in both sexes), cecum (necrosis of epithelial cells in crypts of the cecum in 1 of 10 male), jejunum (hemorrhage in one male), liver (necrosis in 3 of 10 males: necrosis either occurred focally or was seen in scattered hepatocytes throughout liver lobules), adrenal cortex (cytoplasmic vacuolation in 2 of 10 males), lymphoid organs (lymphoid depletion in 6 of 10 males, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 2 of 10 males), spleen (extramedullary hematopoiesis in 2 of 10 animals of each sex), and kidney (tubular necrosis and regenerative changes in the cortex of 8 of 10 males and 1 of 10 females and proteinaceous casts in one male). It is to be mentioned here that males were affected more than females. The histopathological changes in the iron-supplemented animals are shown below.

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Tissue Finding	Males (n = 10)				Females (n = 10)			
	0 mg/kg	100 mg/kg	300 mg/kg	600→ 450 mg/kg	0 mg/kg	100 mg/kg	300 mg/kg	600→ 450 mg/kg
STOMACH								
Erosion, glandular	0	0	0	2	2	2	1	4
Inflammation, glandular	0	0	0	4	0	0	0	3
Ulceration, glandular	0	0	0	1	0	0	0	1
CECUM								
Necrosis, epithelium, crypt	0	0	0	1	0	0	0	0
JEJUNUM								
Hemorrhage	0	0	0	1	0	0	0	0
LIVER								
Necrosis, minimal	0	0	0	2	0	0	0	0
Necrosis, slight	0	0	0	1	0	0	0	0
ADRENAL								
Vacuolation, cortex	0	0	0	2	0	0	0	0
LYMPH NODE								
Mandibular, lymphoid, depletion	0	0	0	2	0	0	0	0
Mandibular, neutrophil infiltrate	0	0	0	1	0	0	0	0
Mesenteric, lymphoid, depletion	0	0	0	2	0	0	0	0
Mesenteric, neutrophil infiltrate	0	0	0	1	0	0	0	0
SPLEEN								
Extramedullary hematopoiesis	0	0	0	2	0	0	0	2
KIDNEY								
Basophilic tubules, cortex	0	0	0	4	0	0	0	1
Cast, tubular	0	0	0	1	0	0	0	0
Mitotic figures, increased	0	0	0	1	0	0	0	0
Necrosis, tubular	0	0	0	7	0	0	0	1

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Standard Diet:

Histopathological changes were observed in the stomach (gastric erosions and/or ulcers and inflammation in males at 100 mg/kg/day and in both sexes at 600 mg/kg/day), cecum (necrosis of epithelial cells in crypts of the cecum in 2 of 10 males and 3 of 10 females at 600 mg/kg/day), liver (necrosis in 8 of 10 males and 2 of 10 females at 100 mg/kg/day, periportal inflammation containing fibrous tissue and bile duct hyperplasia in most males and in one female at 100 mg/kg/day), lymphoid organs (lymphoid depletion in all males at ≥ 100 mg/kg/day and 7 of 10 females at 600 mg/kg/day, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 5 of 10 and 6 of 10 males, respectively, and in 2 of 10 and 3 of 10 females at 600 mg/kg/day, respectively), spleen (extramedullary hematopoiesis in 2 of 9 males at 100 mg/kg/day), heart (multifocal myocytolysis in the ventricle in 4 males and 1 female at 600

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mg/kg/day) and kidney (vacuolation, necrosis and regenerative changes in 9 of 10 males at 100 mg/kg/day and in 1 of 10 females at 600 mg/kg/day and proteinaceous casts in two males at 100 mg/kg/day). It is to be mentioned here that the histopathological changes at 100 mg/kg/day were predominantly observed in males. The histopathological changes in non-iron-supplemented animals are shown below.

Tissue Finding	Males (n = 10)				Females (n = 10)			
	0 mg/kg	100 mg/kg	600 mg/kg		0 mg/kg	100 mg/kg	600 mg/kg	
STOMACH								
Erosion, glandular	0	3	0		1	0	1	
Inflammation, glandular	0	3	2		0	0	1	
Inflammation, nonglandular	0	2	1		0	0	3	
Ulceration, nonglandular	0	2	1		0	0	0	
CECUM								
Necrosis, epithelium, crypt	0	0	2		0	0	3	
LIVER								
Necrosis, minimal	1	4	1		0	2	0	
Necrosis, slight	0	3	0		-	-	-	
Necrosis, moderate	0	1	0		-	-	-	
Inflammation, periportal	0	8	0		0	1	0	
Inflammation, focal	0	1	0		-	-	-	
LYMPH NODE								
Mandibular, lymphoid, depletion	0	3	5		0	0	3	
Mandibular, neutrophil infiltrate	0	0	6		0	0	3	
Mesenteric, lymphoid, depletion	0	4	2		0	0	4	
Mesenteric, neutrophil infiltrate	0	0	5		0	0	2	
SPLEEN								
Depletion, lymphoid	0	7	10		0	0	8	
Extramedullary hematopoiesis	0	2	0		0	0	0	
KIDNEY								
Basophilic tubules, cortex	0	3	0		0	0	0	
Cast, tubular	0	2	0		0	0	0	
Necrosis, tubular	0	8	2		0	0	0	
Vacuolation, tubular	0	1	0		0	0	1	
HEART								
Myocytolysis, multifocal	0	0	0	4	0	0	0	1

Toxicokinetics: In all groups, the drug exposure appeared to be higher in males than in females. The drug did not appear to accumulate over time, as evidenced by comparable AUC_{0-24h} at Day 1 and Day 24 for the low- and mid-dose groups. Based on AUC, the exposure appeared to be higher in animals fed with standard diet than the animals fed with iron-supplemented diet. The

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mean toxicokinetic parameters are shown in the following table (from page 32 of sponsor's submission). There is a trend of higher exposure in males when compared to females. However, the AUC values in the female on Day 1 was calculated based on 0 – 6 hr sampling compared to 0 – 24 hr sampling in males.

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Table 4-3 Mean toxicokinetic parameters of total ICL670

Time	Dose (mg/kg)	Male mice (iron supplemented diet)			Female mice (iron supplemented diet)		
		C _{max}	t _{max}	AUC(0-24h)	C _{max}	T _{max}	AUC(0-24h)
Day 1	100	80.9	2	297	46.8	2	180
	300	196	2	1500	216	2	741*
	600	277	2	4280	288	2	2840
		Male mice (standard diet)			Female mice (standard diet)		
	100	85.7	2	763	81.7	2	618
	600	276	6	5500	300	2	4530
		Male mice (iron supplemented diet)			Female mice (iron supplemented diet)		
Week 4	100	58.2	2	220	44.6	2	184
	300	214	2	1080	195	2	722
	450**	220	2	2020	156	0.5	1170
		Male mice (standard diet)			Female mice (standard diet)		
	100	NS	NS	NS	88.2	2	662
	600	NS	NS	NS	NS	NS	NS

*AUC (0-6h) – no 24 hour samples

**Dose decrease beginning on day 8.

NS = no samples due to early death/sacrifice of the animals.

C_{max} (µg/mL); t_{max} (h); AUC_(0-24h) (µg.h/mL).

Tissue iron analysis: Mean liver iron content in the iron-supplemented animals was approximately 70%, 85%, 82% lower in the males at 100, 300 and 600→450 mg/kg/day compared to respective controls. In females, mean liver iron content in the iron-supplemented animals were approximately 43 – 60% lower in females compared to respective controls at 300 – 600 mg/kg/day. Mean kidney iron content in iron-supplemented males was 60%, 66%, and 50% of control at 100, 300 and 600→450 mg/kg/day. Mean kidney iron content in iron-supplemented females was 90%, 54%, and 42% of control at 100, 300 and 600→450 mg/kg/day.

Summary of individual study findings: In this study, iron-supplemented C57BL/6 mice were treated with ICL-670 by oral gavage at 100, 300 and 600→450 mg/kg/day. The animals with standard diet were treated at 100 and 600 mg/kg/day. The MTD in iron-supplemented males appeared to be 200 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day and severe iron depletion (85%) at ≥ 300 mg/kg/day. However, in case of females, the MTD may be considered as 300 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day. Although iron depletion was also observed in the liver and kidney at

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300 mg/kg/day in females, however, the extent of this iron depletion is much more severe in males when compared to females (43% iron depletion in the liver in females compared to 83% in males at 300 mg/kg/day). In case of animals with standard diet, the MTD in males appeared to be < 100 mg/kg/day based on the mortality and histopathological changes at 100 mg/kg/day. However, in case of females, the MTD may be considered as 100 mg/kg/day based on the mortality at 600 mg/kg/day and absence of any significant histopathological changes at 100 mg/kg/day in females. The target organ of toxicity appeared to be the stomach, liver, lymphoid organs, spleen, kidney and heart (in case of animals fed on standard diet). Overall, it appears that the animals fed on standard diet are more sensitive to the toxicity of ICL-670 than animals fed on iron-supplemented diet, as evidenced by the higher MTD in iron-supplemented animals compared to non-iron-supplemented animals. In addition, there appears to be an apparent sex-related difference in sensitivity to ICL-670. Males appeared to be more sensitive to the toxic effects of ICL-670 compared to females.

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Study Title: A Subchronic Oral (Gavage) Toxicity Study in Juvenile Mice

Key Study Findings: In an oral toxicity study in juvenile mice, animals were treated with ICL670 by oral gavage at 5, 15 and 35 mg/kg/day (with or without an iron-supplemented diet) for up to 5 consecutive weeks from Day 7 *post partum*. The target organs of toxicity appeared to be the thymus (atrophy and necrosis). ICL670 at 35 mg/kg/day caused decreases in B and T-cell populations in the spleen and decreases in T-cell populations in the thymus. The recovery animals still showed these changes and the levels in the blood did not return to control levels. However, the anti-KLH IgM and anti-KLH IgG immune responses were not affected by ICL670 treatment. The NOAEL was considered as 5 mg/kg/day. It appeared that the treatment-related changes were less pronounced in the iron-supplemented animals.

Report No/Study No: 0480148

Volume #, and Page #: EDR/toxicology/0480148

Conducting Laboratory and Location: ☐
J

Date of Study Initiation: October 20, 2004

Date of Study Completion: June 23, 2005

GLP Compliance: The sponsor submitted a draft report of this study under original submission, which did not contain the signed GLP statement. The sponsor then submitted the audited final report of this study under NDA 21-882/000/BP dated July 8, 2005. The results and interpretation of the draft report do not seem to differ from that of the original report. The audited final report is reviewed here.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: ICL-670, Batch No. 0214013, ☐ J

Formulation/Vehicle: Suspension/0.5% (w/v) hydroxypropylcellulose (Klucel HF) aqueous solution

Methods: In this study, the neonatal/juvenile animal was administered ICL670 from postnatal day (PND) 7 through PND 41. ICL670 was administered orally by gavage as suspensions in 0.5% (w/v) hydroxypropylcellulose (Klucel) to four groups of pups at 5 (with iron supplemented diet), 15 (with iron-supplemented diet) and 35 mg/kg/day (with and without iron supplemented diet). An additional 2 groups received an equivalent dosing volume (2 mL/kg) of 0.5% Klucel (one group with and one group without iron supplemented diet) and served as the controls.

The study was divided into three phases to examine toxicology endpoints, immunological endpoints and toxicokinetics. In the toxicology phase, 10 animals/sex/timepoint were

treated for 7, 14 or 35 consecutive days. A fourth timepoint was included and served as a recovery group (35-day treatment plus 28-day recovery). For these animals, clinical signs, mortality, body weight and food consumption were recorded and clinical laboratory (except Day 14 *post partum*) and necropsies with histological review of protocol-defined tissues were performed. For immunological investigations, phenotyping was performed on blood, thymus and spleen on 10 animal/sex/group after 14, and 35 days of treatment. A third timepoint was included and served as a recovery group (35-day treatment plus 28-day recovery). An additional 10 animals/group/sex were included for immunofunctional assessment with keyhole limpet hemocyanin (KLH) challenge at the end of the treatment (35-day treatment) and recovery. Toxicokinetics samples were collected after the 1, 7 and 35 days of administration.

Dosing:

Species/Strain: Mice/CD-1

No./Sex/Group (Main Study): 40/sex/group

Satellite Groups used for Toxicokinetics or Recovery: Toxicokinetics: 108/sex/group; Recovery: 10/sex/group

Age: Postnatal day 7 (PND7)

Weight: 3.0-6.5 g

Doses in Administered Units:

- Group 1: Control (non-supplemented)
- Group 2: Control (Fe-supplemented)
- Group 3: 5 mg/kg/day (Fe-supplemented)
- Group 4: 15 mg/kg/day (Fe-supplemented)
- Group 5: 35 mg/kg/day (Fe-supplemented)
- Group 6: 35 mg/kg/day (Non-supplemented)

Study Design

The study design is shown in the following Tables (from page 23, 24 and 25 of the study report):

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Table 3-1 Phase I – Toxicology phase

Group no. Identification	Dose level (mg/kg/day)	Number of litters	Number of pups ¹	
			males	females
1 Control (non-supplemented)	0	10	40	40
2 Control (Fe supplemented)	0	10	40	40
3 Low (Fe supplemented)	5	10	40	40
4 Mid (Fe supplemented)	15	10	40	40
5 High (Fe supplemented)	35	10	40	40
6 High (non-supplemented)	35	10	40	40

¹ excluding dosed spare pups

On Day 7 *post partum*, a total of 40 pups per sex per group were randomly selected and were allocated into subgroups.

Table 3-2 Phase I - Allocation to subgroups

Sub- group	Duration of dosing	Recovery Period	Subgroup investigations	Number of animals/group ²	
				males	females
A	7 days	N/A	necropsy and histopathology	10	10
B	14 days	N/A	laboratory investigations, necropsy and histopathology	10	10
C	35 days	N/A	laboratory investigations, necropsy and histopathology	10	10
D	35 days	28 days	laboratory investigations, necropsy and histopathology	10	10

² A maximum of one pup per sex per litter was assigned to a given subgroup. This allocation was effective as of Day 7 *post partum*. Additional spare pups were also treated and assigned to a subgroup, when required. The data collected from treated spare animals remaining unassigned to a subgroup by the end of the treatment period were retained with the raw data but were not reported.

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Table 3-3 Phase II – Immunology phase

Group no. Identification	Dose level (mg/kg/day)	Number of litters	Number of pups ¹	
			males	females
1 Control (non-supplemented)	0	14	50	50
2 Control (Fe supplemented)	0	14	50	50
3 Low (Fe supplemented)	5	14	50	50
4 Mid (Fe supplemented)	15	14	50	50
5 High (Fe supplemented)	35	14	50	50
6 High (non- supplemented)	35	14	50	50

¹ excluding dosed spare pups

On Day 7 *post partum*, a total of 50 pups per sex per group were randomly selected and were allocated into subgroups.

Table 3-4 Phase II - Allocation to subgroups

Sub- group	Duration of dosing	Recovery period	Subgroup investigations	Number of animals/group ²	
				males	females
E	14 days	N/A	phenotyping	10	10
F(i)	35 days	N/A	phenotyping	10	10
F(ii)	35 days	N/A	antibody response	10	10
G(i)	35 days	28 days	phenotyping	10	10
G(ii)	35 days	28 days	antibody response	10	10

² A maximum of one pup per sex per litter was assigned to a given subgroup. This allocation was effective as of Day 7 *post partum*. Additional spare pups were also treated and assigned to a subgroup, when required. The data collected from treated spare animals remaining unassigned to a subgroup by the end of the treatment period were retained with the raw data but were not reported.

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Table 3-5 Phase III – Toxicokinetic phase

Group no. Identification	Dose level (mg/kg/day)	Number of litters	Number of pups ¹	
			males	females
1 Control (non-supplemented)	0	16	54	54
2 Control (Fe supplemented)	0	16	54	54
3 Low (Fe supplemented)	5	28	108	108
4 Mid (Fe supplemented)	15	28	108	108
5 High (Fe supplemented)	35	28	108	108
6 High (non- supplemented)	35	28	108	108

¹ excluding dosed spare pups

On Day 7 *post partum*, litters were randomly selected and were allocated into subgroups.

Table 3-6 Phase III - Allocation to subgroups

Sub- group	Duration of dosing	Subgroup investigations	Number of animals/group ²	
			males	females
H++	1 day	single dose	Control: 6+	Control: 6+
		3 pooled samples/sex/time point; 4 time points for ICL670 designated animals and 2 time points for controls+	ICL670: 12+	ICL670: 12+
I	7 days	3 pooled samples/sex/time point; 4 time points for ICL670 designated animals and 2 time points for controls+	Control: 6+	Control: 6+
		4 time points for ICL670 designated animals and 2 time points for controls+	ICL670: 12+	ICL670: 12+
J	35 days	3/sex/time point; 4 time points for ICL670 designated animals and 2 time points for controls+	Control: 6+++	Control: 6+++
		4 time points for ICL670 designated animals and 2 time points for controls+	ICL670: 12+++	ICL670: 12+++

* A pooled sample from 4 pups (when possible) of the same sex within the litter was used to obtain the required volume.

** Ten additional litters per group were assigned to the subgroup H following changes to the blood sample processing procedures (see Section 3.9).

+++ A maximum of one pup per sex per litter was assigned to a given timepoint.

² Additional spare pups were also treated and assigned to subgroup I or J, when required. The data collected from treated spare animals remaining unassigned to a subgroup by the end of the collection period were retained with the raw data but were not reported.

Basis of Dose Selection: Doses of 5, 15 and 35 mg/kg/day were selected based on the results of the oral juvenile development dose range-finding study in mice (Study 0470147). In this dose ranging study, ICL670 was administered orally by gavage as suspensions in 0.5% (w/v) hydroxypropylcellulose (Klucel) to four groups of juvenile (PND 7) mice (12 pups/sex/group) at 10, 20, 40 or 60 mg/kg/day. An additional group (12 pups/sex) received an equivalent dosing volume (2 mL/kg) of 0.5% Klucel and served as the controls. All groups received iron supplemented feed and were treated once daily on post partum days 7 through 41. Due to mortality and moribundity, the high dose (60 mg/kg/day) was terminated early on PND 14 to 16. At 40 mg/kg/day, approximately 33% of the males and 25% of the females died between PND 15 and 18. There was no

mortality following PND 18. Overall, ICL670 produced mortality in juvenile mice at ≥ 40 mg/kg/day. The NOAEL was considered as 20 mg/kg/day. A high dose of 35 mg/kg/day was considered to be suitable for the current juvenile study in mice.

Route, Form, and Dose Volume: Oral (gavage), suspension, 2 ml/kg

Observations and Times:

Mortality: All dams and pups were examined twice daily for mortality.

Clinical Signs: Clinical signs were examined twice daily.

Body Weights: All dams were weighed on Days 0, 4, 7, 14, 17 and 21 post partum. For F1 pups, body weights were measured on PND 7-21, and on Days 24, 28, 31, 35, 38 and 42 *post partum*, then twice weekly until terminal euthanasia.

Food Consumption: Food consumption for Phase 1 animals was measured weekly after weaning from Days 21, 24, 28, 31, 35, 38 and 42 *post partum* then twice weekly until terminal euthanasia.

Hematology: Blood samples were collected for hematology from Phase 1 animals. Blood samples were collected from subgroups E to G, Subset i, for phenotyping and from subgroups F and G, Subset ii, for antibody response.

Clinical Chemistry: Blood samples were collected for serum chemistry from Phase 1 animals at necropsy.

Gross Pathology: Gross pathology was conducted at necropsy (subgroups A-D).

Organs Weighed: The following organs were weighed from all animals in subgroups B to G: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, and parathyroid.

Histopathology: The following organs/tissues (from page 31 of the study report) were examined for histopathology from all animals in subgroups A, B, C and D:

Subgroups A, B, C and D

abnormalities	ileum	sciatic nerve
animal identification ^a	jejunum	seminal vesicles
adrenals	kidneys	skeletal muscle
aorta (thoracic)	lacrimal glands	skin (inguinal)
bone and marrow ^{**} (sternum)	liver (sample of 2 lobes)	spinal cord (cervical)
brain (cerebrum, cerebellum, midbrain and medulla oblongata)	lungs ⁺⁺	spleen
cecum	lymph nodes (mandibular and mesenteric)	stomach
colon	mammary gland ⁺ (inguinal)	testes [*]
duodenum	optic nerves ^{+,***}	thymus
epididymides [*]	ovaries	thyroid lobes [*] (and parathyroids)
esophagus	pancreas	tongue
eyes ^{***}	pituitary	trachea
femur ^{**}	prostate	urinary bladder
gall bladder	rectum	uterus (horns, body and cervix)
Harderian glands	salivary gland	vagina
heart (including section of aorta)		

Toxicokinetics: (Subgroups H, I, J) After a single dose on Day 7 *post partum*, 3 litters of 4 pups/sex/group/time point were bled at 0.5 and 2 hours (control groups) and 0.5, 2, 6 and 24 hours (ICL670 groups) after administration of treatment. Day 13 *post partum*, 3 litters of 4 pups/sex/group/time point were bled at 0.5 and 2 hours (control groups) and 0.5, 2, 6 and 24 hours (ICL670 groups) after administration of treatment. On Day 41 *post partum*, 3 mice/sex/group/time point (from different litters) were bled at 0.5 and 2 hours (control groups) and 0.5, 2, 6 and 24 hours (ICL670 groups) after administration of treatment.

Phenotyping: (Subgroups E, F and G, subset i). Phenotyping of the spleen and thymus was performed. Phenotyping results were reported as relative proportions and absolute numbers of CD4⁺, CD8⁺, CD3⁺, CD19⁺, NK1.1, CD8⁺/CD44^{hi}/Ly-6C⁺/CD62L⁻ (CD8 memory T-cell marker), CD4⁺/CD44^{hi}/CD62L⁻ (CD4

memory T-cell marker), and CD3/NK1.1/F4/80 (mac)/CD19/CD45R/B220 (memory B-cells) lymphocyte subsets. The antibodies used were as follows:

- CD3/CD4/CD8 (blood, spleen and thymus)
- CD3/CD19/NK 1.1 (blood and spleen)
- CD4/C44^{hi}/CD62L⁻ (blood and spleen)
- CD8/CD44^{hi}/CD62L⁻/Ly-6C⁺ (blood and spleen)
- F4/80/NK1.1/CD3/CD19/CD45R/B220 (spleen only)

T-Cell Dependent Antibody Response Using KLH (Subgroups F and G, subset ii):

The T-cell dependant antibody response was monitored using 10 animal/sex/group from subgroups F and G, subset ii. The anti-KLH IgM and anti-KLH IgG response was monitored for each animal from these subgroups at the time points indicated in the following table (from page 35 of the study report). Briefly, blood samples were collected from a jugular vein for immunology. Blood samples were centrifuged and the serum aliquoted and stored at approximately -70°C until analysis. KLH solution (0.5 mL; 0.2 mg/mL solution) was administered by intraperitoneal injection at the following time points and after blood collection for immunoglobulin measurements.

Age (days post partum)	28	35	42	56	63	70
F	KLH	Ig, KLH	Ig			
G		Ig (baseline)		KLH	Ig, KLH	Ig

Results:

Mortality: Eleven (one female at 15 mg/kg/day and 9 males and 2 females at 35 mg/kg/day) ICL670-treated mice were found dead between Days 12 and 25 *post partum* and one male was euthanized in poor condition on Day 19 *post partum* (pp). Clinical signs in these animals included: dehydration, eyes partly closed (ptosis), uncoordination, hunched posture, labored breathing, skin palor and/or animal lying on its side, weak or thin. The cause of death could not be determined at gross pathological examination. Treatment-related microscopic changes were observed in the liver (pigment deposits) and thymus (lymphoid necrosis/atrophy). Overall, dose-related incidence was as follows: 1 mouse at 15 mg/kg/day, 4 mice were at 35 mg/kg/day (iron-supplemented diet) and 7 mice were at 35 mg/kg/day (non iron-supplemented diet). Two additional animals (iron supplemented control female and non-iron-supplemented high dose male) were also found dead due to gavage accident on Day 24 and Day 32 *post partum*,

respectively. In addition, another non-supplemented control female died on Day 23 pp. The cause of death was unknown.

Clinical Signs: The following clinical signs were observed in few pups at 35 mg/kg/day: dehydration, ptosis, animal thin or weak, hunched posture, breathing labored, activity decreased, and/or piloerection. There were no further changes attributed to treatment in the high-dose groups from Day 36 pp onwards. In addition, a few other signs observed of low incidence included skin scab, black or blue skin on the tail, red liquid discharge from the mouth, fur ungroomed. These signs were also observed in the control group pups and were considered incidental or secondary to the technical procedures (tattooing and gavage procedures, respectively).

Body Weights: The mean initial and final body weight of control male pups were 1.62 and 12.97 g, respectively. The mean initial and final body weight of control female pups were 1.55 and 12.47g, respectively. The final body weights of control male pups were 95.8, 96.9, 95.9, 91.0 and 85.9% of control in Group 2, 3, 4, 5, and 6, respectively. The final body weights of treated female pups were 93.9, 95.5, 95.4, 89.9 and 84.4% of control in Group 2, 3, 4, 5 and 6, respectively.

Food Consumption: The mean initial and final food consumption in control male pups were 1.5 and 1.0 g/animal/day, respectively. The mean initial and final food consumption in control female pups were 1.0 and 1.0 g/animal/day, respectively. During the post-weaning treatment period, the food intake of litters treated at each dose level was generally lower compared to controls from Days 21 to 24 and Days 24 to 28 pp. Thereafter, the individual food intake was comparable to the iron supplementation and/or non-supplementation control groups. During the recovery period, the food intake at each dose level was unaffected.

Hematology: On Days 21 and 42 pp, and at the end of the recovery period, on Day 70 pp, the absolute white blood cell, lymphocyte and neutrophil counts were reduced marginally in non iron-supplemented males at 35 mg/kg/day when compared to the corresponding controls. There were no other consistent significant treatment-related changes.

Clinical Chemistry: No significant treatment-related changes were observed.

Organ Weights: Treatment-related decrease in the absolute and relative (to body and brain weights) thymic weight was observed at Day 21 pp in male (absolute: 60% of control, control = 0.05 g) and female mice (absolute: 60% of control, control = 0.049 g) at 35 mg/kg/day, with or without iron supplementation, when compared to mice in either of the two control groups. There were no ICL670-related organ weight changes seen following the 35-day treatment period and 28-day recovery period.

Gross Pathology: There were no significant treatment-related changes.

Histopathology: No treatment-related histopathological changes were observed at Day 14. However, treatment-related histopathological changes were seen in the following organs at Day 21 at 35 mg/kg/day: liver (slight to moderate pigment deposits), thymus (increased severity of lymphoid necrosis/atrophy). In the liver, pigment deposits were seen in a few male and female mice in both the iron-supplemented and the non-supplemented groups but with an apparent increase in incidence and severity in the non-supplemented group. In the thymus, increased severity of lymphoid necrosis/atrophy, when compared to the control groups, was evident in the group without iron supplementation and correlated to the 30 to 40% decrease in thymus weights noted at necropsy and the decreased lymphocyte populations seen at the immunological assessment. This was not observed in the iron-supplemented group. At day 42 pp, there was no evidence of ICL670-related increased severity of thymic lymphoid necrosis/atrophy at 35 mg/kg/day. Relevant histopathological changes in the preterminal animals are shown in the following table.

MALE:

Tissue Finding	Grade	Males (Groups)					
		1	2	3	4	5	6
Epididymides: Oligo/aspermia	1						
	2						
	3						1/7
	4						
	5					2/2	6/7
Esophagus: inflammation	1						
	2						1/2
	3						1/2
	4						
Heart: Inflammation, epicardial	1						1/1
	2						
	3						
	4						
	5						
Kidney: tubular basophilia	1						1/1
	2						
	3						
	4						
	5						
Liver: pigment deposit	1						1/4
	2					1/2	3/4
	3					1/2	
	4						
	5						
Lung: hemorrhage	1					1/2	
	2					1/2	3/3
	3						

	4						
	5						
Thymus: atrophy/necrosis	1						
	2						
	3					2/2	3/3
	4						
	5						

FEMALE:

Tissue Finding	Grade	Males (Groups)					
		1	2	3	4	5	6
Esophagus: degeneration/regeneration	1						
	2						
	3		1/1				
	4						
Kidney: tubular basophilia	1				1/1		
	2						
	3						
	4						
	5						
Liver: pigment deposit	1						
	2						1/1
	3					1/1	
	4						
	5						
Lung: hemorrhage	1						
	2						
	3						1/1
	4						
	5						
Thymus: atrophy/necrosis	1						
	2						
	3						1/1
	4						
	5						

- GROUP 1: Control (non-supplemented)
- GROUP 2: Control (Fe supplemented)
- GROUP 4: Mid (Fe supplemented) 15 mg/kg/day
- GROUP 5: High (Fe supplemented) 35 mg/kg/day
- GROUP 6: High (non-supplemented) 35 mg/kg/day

- GRADE 1 = Minimal/very few/very small
- GRADE 2 = Slight/few/small
- GRADE 3 = Moderate/moderate number/moderate size
- GRADE 4 = Marked/many/large

GRADE 5 = Severe/extensive number/extensive size

Toxicokinetics: Based on the dose normalized AUC_{0-24h} values, the exposure appeared to be dose proportional between 5 and 35 mg/kg/day in iron supplemented males and females on Days 7/8 and 13/14 pp and it was greater than dose proportional on Days 41/42 pp. At 35 mg/kg/day, the exposure was comparable on Days 7/8 and 13/14 pp across both groups however, on Days 41/42 pp, the exposure appeared to be higher (5 to 7- fold) in non-supplemented animals than iron supplemented animals. There was no apparent difference in exposure between males and females in each group. The toxicokinetic parameters are shown in the following table (from page 42 of the study report).

Table 4-1 Toxicokinetic parameters of total ICL670 in mouse plasma

Days 7/8 post partum

Parameter (units)	Low dose + Fe (5 mg/kg/day)		Mid dose + Fe (15 mg/kg/day)		High dose + Fe (35 mg/kg/day)		High dose (35 mg/kg/day)	
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	6.0	2.0	2.0	2.0	6.0	2.0	2.0	2.0
C_{max} (µg/mL)	1.86	2.29	6.44	4.97	14.4	11.2	11.6	14.2
$C_{max}/Dose$ (µg/mL)/(mg/kg/day)	0.372	0.458	0.429	0.331	0.411	0.320	0.331	0.408
AUC_{0-24h} (µg.h/mL)	25.3	22.6	98.6	76.4	214	147	145	139
$AUC_{0-24h}/Dose$ (µg.h/mL)/(mg/kg/day)	5.06	4.52	6.57	5.09	6.11	4.21	4.14	3.95

Days 13/14 post partum

Parameter (units)	Low dose + Fe (5 mg/kg/day)		Mid dose + Fe (15 mg/kg/day)		High dose + Fe (35 mg/kg/day)		High dose (35 mg/kg/day)	
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	2.0	2.0	2.0	2.0	2.0	2.0	6.0	2.0
C_{max} (µg/mL)	4.93	5.85	20.4	16.9	45.3	35.3	39.7	35.9
$C_{max}/Dose$ (µg/mL)/(mg/kg/day)	0.986	1.17	1.36	1.13	1.29	1.01	1.13	1.03
AUC_{0-24h} (µg.h/mL)	55.8	72.9	205	232	652	461	547	523
$AUC_{0-24h}/Dose$ (µg.h/mL)/(mg/kg/day)	11.2	14.6	13.7	15.5	18.6	13.2	15.5	15.0

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Days 41/42 post partum

Parameter (units)	Low dose + Fe (5 mg/kg/day)		Mid dose + Fe (15 mg/kg/day)		High dose + Fe (35 mg/kg/day)		High dose (35 mg/kg/day)	
	Males	Females	Males	Females	Males	Females	Males	Females
$t_{1/2}$ (h)	0.5	2.0	0.5	0.5	0.5	0.5	0.5	2.0
C_{max} ($\mu\text{g/mL}$)	0.203	0.113	0.635	1.87	5.96	3.74	18.9	14.1
C_{max}/Dose ($\mu\text{g/mL}/(\text{mg/kg/day})$)	0.0406	0.0226	0.0423	0.125	0.159	0.107	0.540	0.403
$AUC_{0-24\text{h}}$ ($\mu\text{g}\cdot\text{h/mL}$)	0.203	0.369	4.48	8.05	17.6	14.7	81.8	105
$AUC_{0-24\text{h}}/\text{Dose}$ ($\mu\text{g}\cdot\text{h/mL}/(\text{mg/kg/day})$)	0.0406	0.0776	0.299	0.537	0.502	0.420	2.34	3.00

Phenotyping: When considering the different lymphocyte populations in the blood, spleen and thymus on Days 21, 42 and 70 *post partum*, only a very few statistically significant differences in the cell counts were observed, when compared to the respective controls, and occurred predominantly in the non iron-supplemented males at 35 mg/kg/day. When compared to respective controls, Band T-cell lymphocyte populations were typically increased in the blood on Days 21 and 42 pp in non iron-supplemented animals at 35 mg/kg/day and some lymphocyte populations, notably B- cells, were increased on Day 21 pp in iron-supplemented animals at each dose level. In addition, on Day 21 pp at 35 mg/kg/day with or without iron supplementation, there were decreases in B and T-cell populations in the spleen and decreases in T-cell populations in the thymus followed by a recovery on Day 42 pp in the thymus only. The recovery animals still showed these changes and the levels in the blood did not return to control levels. However, there was no apparent difference in these responses across sexes.

T-Cell Dependent Antibody Response: The anti-KLH IgM and anti-KLH IgG immune responses were not affected by ICL670 treatment.

Summary: In an oral toxicity study in juvenile mice, animals were treated with ICL670 by oral gavage at 5, 15 and 35 mg/kg/day (with or without an iron-supplemented diet) for up to 5 consecutive weeks from Day 7 *post partum*. The target organ of toxicity appeared to be the thymus (atrophy and necrosis). ICL670 at 35 mg/kg/day caused decreases in B and T-cell populations in the spleen and decreases in T-cell populations in the thymus. The recovery animals still showed these changes and the levels in the blood did not return to control levels. However, the anti-KLH IgM and anti-KLH IgG immune responses were not affected by ICL670 treatment. The NOAEL was considered as 5 mg/kg/day. It appeared that the treatment-related changes are less pronounced in the iron-supplemented animals. It is to be mentioned here that the sponsor did not conduct ophthalmoscopic examinations.

Exploratory Comparative 4-Week Oral Toxicity Study in Rats (Study No. 956214)

Methods: This study was performed to obtain information about the oral toxicity of iron chelators in rats at an early stage. In this exploratory study, the toxicity of 4 different substances of new classes of potential iron chelators was examined in rats. In this study, female rats [Tif:RAIf (SPF)] were administered orally (gavage) ICL670 at 135 and 270 mg/kg/day for 4 consecutive weeks at a dose volume of 10 ml/kg in Klucel 0.5%. In addition other groups received three other different substances as shown in the following table (from page 5 of the study report). The present review will restrict to ICL670 only.

Group	Compound	Chemical Name	Dose (mg/kg)
1	Control Article	Klucel HF	0
2	CGP 65 015	Hydroxypyridinone	140
3			280
4	CGP 69 939	Bis-hydroxyphenyltriazole	270
5			540
6	CGP 72 670	Bis-hydroxyphenyltriazole	135
7			270
8	CGP 72 671	Bis-hydroxyphenyltriazole	220
9			440

Clinical signs and mortality was observed on daily basis. Body weights were recorded 3 times per week and thereafter on a daily basis. Hematology, clinical chemistry and urinalysis were performed on Week 1 and 4. The following organs were weighed at necropsy: kidneys, liver, spleen and thymus. The following tissues were examined histopathologically from all treated animals: kidneys, liver, small intestine, spleen, sternum, and thymus.

Results: One rat at 270 mg/kg/day was sacrificed on moribund condition at Week 3. The cause of death was attributed to the kidney or liver toxicity. Clinical signs at 270 mg/kg included sunken flanks of a slight degree (persistent as of Week 2) and reddish discoloration of feces (transient) in all rats. In addition, decreased activity, stiff gait, hunched posture, piloerection, diarrhea and muscular hypotonia of a slight to moderate degree were transiently seen in individual animals. Slightly but transiently reduced body weight gain was seen at both doses. There were decreases in cholinesterase (high-dose) and triglycerides (dose-related). At 270 mg/kg, reticulocyte and thrombocyte counts were reduced in Week 1. At the end of treatment, thrombocyte counts and segmented

neutrophils were slightly increased. Morphological changes in red blood cells were seen, which consisted of anisocytosis, hypochromasia, microcytosis accompanied by the presence of normoblasts. In addition, slight oliguria was observed in rats at all doses. There were no effects on organ weights. Discoloration of the kidneys (pale) was noted in one animal at 270 mg/kg. Histopathological findings included moderate tubular degeneration in the kidneys and marked thymus atrophy at 270 mg/kg. The 135 mg/kg/day dose was considered as NOAEL. The target organ of toxicity appeared to be the kidney.

4-Week Oral Experimental Toxicity Study in Rats (Study No. 971055)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: November 4, 1997

Date Completed: February 6, 1998

GLP Compliance: A statement of compliance was included.

Animals: Wistar Hannover (— WIGA) rats were used throughout the study. Animals were 20 weeks old at the start of the dosing and body weight ranges from 293-439 g for males and 190-242 g for females.

Drug Batch: ICL 670, Batch No. 800196.

Methods: The purpose of this oral toxicity study in iron-overloaded and non-iron-overloaded rats was to test the hypothesis that the nephrotoxic effects of ICL 670 were largely due to its pharmacological action (massive iron depletion). Iron overloading was done by a twice-weekly intraperitoneal injection of 0.2 ml/kg Anaemex[®] for 6 weeks during the pretest period. Iron-overloaded and non-overloaded rats (10/sex/group) were administered by oral gavage (5 ml/kg) with 100 mg/kg/day of ICL 670 (2% ICL 670 in 0.5% Klucel HF) for approximately 2 weeks (on a 7 days a week dosing schedule) for interim sacrifice and up to 4 weeks for terminal sacrifice. Clinical signs were recorded daily. Body weights and food consumption were recorded once per week during the pretest and treatment periods. Hematology and clinical chemistry were performed 2 days prior to necropsy. Organ weights were taken for adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus. Microscopic examinations were conducted on adrenals, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, Harderian glands, heart, ileum, jejunum, kidneys, knee joint, lacrimal glands, liver, lungs, lymph nodes, mammary gland, ovaries, pancreas, peripheral nerves, pituitary, prostate, rectum, salivary glands, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroids, tongue, trachea, urinary bladder, uterus, and vagina.

Results:

1. **Clinical Signs:** Major clinical signs in the non-iron-overloaded rats included sedation, ptosis, piloerection, and red discoloration of feces, diarrhea, labored breathing, and chromorrhinorhea in males while females remain unaffected.

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2. **Mortality:** Four deaths were reported in the non-iron-overloaded rats and deaths occurred on Day 11. There were no deaths in the iron-overloaded rats.
3. **Body Weight:** The mean initial and final body weights of control males were 256 and 364 g, respectively. The mean initial and final body weights of control females were 179 and 223 g, respectively. The body weight of male rats in group 5 (100 mg/kg, non-iron-overloaded rats) was significantly reduced (81% of control) during week 2 compared to control (369 g). In case of females, body weights were significantly reduced in both iron-overloaded (92% of control) and non-iron-overloaded (91% of control) rats at week 4 compared to control.
4. **Food Consumption:** The mean initial and final food consumption in control males were 168 and 158 g/week, respectively. The mean initial and final food consumption in control females were 115 and 123 g/week, respectively. The food consumption was significantly reduced in males at week 2 in both iron-overloaded (85% of control) and non-iron-overloaded (39% of control) rats compared to control (153 g/week). In the females, the food consumption was reduced at week 4 in both iron-overloaded (94% of control) and non-iron-overloaded (93% of control) rats.
5. **Hematology:** Zinc protoporphyrin (ZPP) values were significantly increased in males (174% in males of control, control = 68.8 $\mu\text{mol/mol}$) and in females (154% of control, control = 61 $\mu\text{mol/mol}$) in non-iron-overloaded rats on study day 16. RBC (124% of control, control = 9.01 $\text{mi}/\mu\text{l}$), hematocrit (120% of control, control = 47.9%), and hemoglobin (121% of control, control = 15.4 g/100 ml) values were increased in non-iron-overloaded males at day 16 of the study. At study day 30, the ZPP values were still elevated (160% of control, control = 65 $\mu\text{mol/mol}$) in non-iron-overloaded males. Reticulocytes were also increased in both iron-overloaded (254% of control, control = 2.4% RBC) and non-iron-overloaded (229% of control, control = 2.4% RBC) male rats at study day 30. In the non-iron-overloaded females, hemoglobin (89% of control, control = 15.7 g/100 ml), mean corpuscular volume (93% of control, control = 56 fl), mean corpuscular hemoglobin (89% of control, control = 17.9 pg) were decreased at day 30. The reticulocyte values were increased in both iron-overloaded (154% of control, control = 2.8% RBC) and non-iron-overloaded (171% of control, control = 2.8% RBC) female rats at day 30.
6. **Clinical Chemistry:** Total protein (105% of control, control = 66 g/L), albumin (109% of control, control = 34 g/L), urea (132% of control, control = 5.8 mmol/L), creatinine (140% of control, control = 50 $\mu\text{mol/L}$), total bilirubin (143% of control, control = 7.2 $\mu\text{mol/L}$), cholesterol (175% of control, control = 0.8 mmol/L), magnesium (122% of control, control = 0.93 mmol/L) values were altered significantly on day 16 in iron-overloaded males. On day 30, creatinine (155% and 127% of control in non-iron-overloaded and iron-overloaded rats, respectively, control = 49 $\mu\text{mol/L}$), cholesterol (212% and 175% of control in non-iron-overloaded and iron-overloaded rats, respectively), total bilirubin (132% of control, control = 6.8 $\mu\text{mol/L}$), sodium (104% of control, control = 146 mmol/L), phosphorus (129% of control, control = 146 mmol/L), and magnesium (137% of control, control = 1.04 mmol/L), chloride (106-107% of control in both the groups, control = 116 mmol/L), cholinesterase activity (180 and 143% of control in non-iron overloaded and iron-overloaded rats) values were altered in male rats. In the females, total protein (107 and 108% of control in iron-overloaded and non-iron overloaded rats, respectively, control = 72 g/L), albumin (110-111%

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of control in both the groups, control = 36 g/L), creatinine (117-118% of control in both the groups, control = 56 µmol/L), creatinine kinase (61% and 70% of control in non-iron-overloaded and iron-overloaded rats, respectively, control = 314 IU/L), total bilirubin (124% of control in non-iron-overloaded rats, control = 7.9 µmol/L), cholesterol (166% and 217% in iron-overloaded and non-iron-overloaded rats, respectively, control = 0.6 mmol/L), triglyceride (145% of control in non-iron-overloaded rats, control = 0.38 mmol/L), phosphorus (118% of control in non-iron-overloaded rats, control = 1.32 mmol/L), and magnesium (110% of control in both iron-overloaded and non-iron-overloaded animals, control = 1.20 mmol/L) values were altered at 30 day time point.

7. **Urinalysis:** Urine pH and creatinine clearance values were slightly lowered in iron-overloaded and non-iron-overloaded rats on study days 16 and 30 in both the sexes.
8. **Organ Weights:** No organ weight analysis was available for non-iron-overloaded males. Treatment-related absolute weight changes were observed in thyroid (71% of control, control = 0.017 g), uterus (69% of control, control = 0.71 g), liver (91% of control, control = 7.19 g) and adrenals (73% of control, control = 0.077 g) of the non-iron-overloaded females. Treatment-related relative weight changes were observed in kidney (119% of control, control = 0.669), brain (109% of control, control = 0.882), and adrenal glands (81% of control, control = 0.037) in non-iron-overloaded females. No organ weight changes were noted in iron-overloaded males at the interim sacrifice on day 18. Treatment-related weight changes were observed in kidney (relative, 125% of control, control = 0.652), liver (74% and 89% of control in absolute and relative weights, respectively. Absolute control = 13.73 g and relative control = 3.84), thymus [(57% (absolute) and 68% (relative) of control, control = 0.312 g (absolute) and 0.088 (relative)] and prostate (57% of control, control = 0.662 g) of iron-overloaded males at terminal sacrifice. In the iron-overloaded females, treatment-related changes were observed in kidney (166% of control in relative weight, control = 0.684), liver (85% of control, control = 8.41 g), thymus [68% (absolute) and 77% (relative) of control, control = 0.268 g (absolute) and 0.124 (relative)], and adrenal (84% of control, control = 0.073 g) glands at terminal sacrifice. At the interim sacrifice in iron-overloaded females, treatment-related changes were observed only in the absolute weight of liver (84% of control, control = 12.79 g).
9. **Gross Pathology:** All animals in group 5 (non-iron-overloaded, 100 mg/kg ICI, 670) were either found dead (due to nephrotoxicity) or sacrificed due to poor general condition. Red discoloration of the mesenteric lymph node was observed in several iron-overloaded rats including controls.
10. **Histopathology:** In non-iron-overloaded rats, treatment-related toxicities were seen at 100 mg/kg in the kidneys (tubular degeneration, which included renal tubular vacuolation and tubular necrosis), gastrointestinal tract (minimal to moderate non-glandular stomach ulceration, moderate ulcerative typhlitis and slight to moderate colitis), thymus (thymus atrophy), spleen (lymphoid atrophy, less pronounced extramedullary hemopoiesis in treated males compared to control, increased hemosiderosis in treated males), lymph node (lymphoid atrophy in the mandibular and mesenteric lymph node), prostate (epithelial atrophy in all males), uterus (minimal atrophy), and adrenals (cortical atrophy). Males appeared to be more susceptible than females. In iron-overloaded rats, histopathologic alterations were seen at

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100 mg/kg in the kidneys (tubular degeneration in both sexes, which included tubular vacuolation and necrosis). The changes seen in iron-overloaded rats were more severe after 18 days compared to 4 weeks of treatment indicating regeneration and/or adaptation subsequent to toxic injury of (tubular cells), thymus (thymus atrophy), and prostate (epithelial atrophy).

11. **Toxicokinetics:** ICL 670 was administered once a day by oral gavage at dose of 100 mg/kg to iron-preloaded and non iron-preloaded males and female rats. Blood samples were collected on Day 1, 17, and 31 for drug plasma concentration measurements. The toxicokinetic parameters of total ICL 670 in a 4-week oral toxicity study in iron-overloaded and non-iron-overloaded rats are shown below (from vol. 19, page 8-5127 of sponsor's submission):

Iron-preloaded Male Rats					Iron-preloaded Female Rats				
Dose/ mg/kg	Day	Cmax	Tmax	AUC	Dose/ mg/kg	Day	Cmax	Tmax	AUC
100	1	131	2	1060	100	1	134	2	892
100	17*	164	8	1900	100	17	-	-	-
100	31*	118	0.5	811	100	31	43.5	8	619
Non-iron-preloaded Male Rats					Non-iron-preloaded Female Rats				
Dose/ mg/kg	Day	Cmax	Tmax	AUC	Dose/ mg/kg	Day	Cmax	Tmax	AUC
100	1	118	2	1190	100	1	129	2	1300
100	31	-	-	-	100	31	98.1	4	845

*: One male animal/sampling time only.
Cmax (µg/ml); Tmax (hr); AUC (0-24h) (µg.h/ml).

The mean plasma concentrations of total ICL 670 in iron pre-loaded and non iron-preloaded rats on Day 1 were similar in both the sexes. The systemic exposure of female iron-preloaded rats showed a tendency to decrease upon repeated dosing according to the Cmax values.

In a 4-week oral experimental toxicity study in rats, iron-overloaded and non-iron over-loaded rats were treated orally with 100 mg/kg of ICL 670. Four deaths were reported in the non-iron-overloaded rats and deaths occurred on Day 11. There were no deaths in the iron-overloaded rats. No observed effect level was not established. The target organ of toxicity appeared to be kidney (tubular degeneration, which included renal tubular vacuolation and tubular necrosis), gastrointestinal tract in non-iron-overloaded rats (minimal to moderate non-glandular stomach ulceration, moderate ulcerative typhlitis and slight to moderate colitis), thymus (thymus atrophy), spleen in non-iron-over-loaded rats (lymphoid atrophy, less pronounced extramedullary hemopoiesis in treated males compared to control, increased hemosiderosis in treated males), lymph node in non-iron-overloaded rats (lymphoid atrophy in the mandibular and mesenteric lymph node), prostate (epithelial atrophy in all males), uterus in non-iron-overloaded rats (minimal atrophy), and adrenals (cortical atrophy in non-iron-overloaded rats). Males appeared

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to be more susceptible than females in non-iron-overloaded animals. The changes seen in iron-overloaded rats were more severe after 18 days compared to 4 weeks of treatment indicating regeneration and/or adaptation subsequent to toxic injury of tubular cells. The nephrotoxic potential of ICL 670 was less pronounced in iron-over-loaded animals.

4-Week Oral (gavage) Exploratory Study in Male Rats (Study No. 987066)

Testing Laboratory: Novartis Pharmaceuticals Corporation
East Hanover, New Jersey.

Date Started: July 14, 1998

Date Started: August 12, 1998

GLP Compliance: A statement of compliance was included.

Animals: Wistar rats were used throughout the study. Animals were approximately 8 weeks old at the initiation of dosing and body weight ranges from 220-296 g.

Drug Batch: ICL 670, Batch number 97902.

Methods: The purpose of this study was to examine and compare the pharmacologic and toxicologic effects of ICL 670 in male rats fed a standard diet and an iron-supplemented diet (in the form of ferrous sulfate monohydrate (31%) to achieve a total of 1680 ppm iron) and to select doses for an ongoing 26-week oral (gavage) toxicity study in rats. In this study, ICL 670 was administered orally (gavage) to male Wistar rats (10/group) at a daily dose of 100 mg/kg at a dose volume of 10 ml/kg. The doses were selected based on the results of a 4-week oral (gavage) toxicity study in rats with a 4-week recovery period (Study No. 974080). In the 4-week study, mortality was observed at 100 mg/kg/day and caused renal tubular vacuolation and necrosis in the kidney and focal degeneration, inflammation and myocarditis in the heart. In the current study, 100 mg/kg was expected to produce definitive signs of toxicity in the standard/non-iron-supplemented feed. Animals were monitored for clinical signs of toxicity and morbidity/mortality daily. Body weight and food consumption was recorded once weekly. Hematology and clinical chemistry parameters were measured during treatment week 4. Rats were sacrificed at the end of the treatment period and subjected to necropsy examination. The following organ weights were taken at necropsy: liver, spleen, brain, kidneys, thymus, heart, and adrenal. Histopathological examinations were performed on the following organs: liver, esophagus, jejunum, colon, tongue, stomach, ileum, rectum, salivary gland, duodenum, cecum, pancreas, trachea, lungs, eyes, Harderian glands, lacrimal glands, femur, sternum, skeletal muscle, heart, aorta, skin, mammary, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, bone marrow, mesenteric lymph node, mandibular lymph node, thymus, bronchial lymph node, spleen, pituitary, parathyroids, adrenals, thyroid, brain, spinal cord, sciatic nerve, etc.

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Results:

1. **Observed Effects:** Treatment-related clinical signs were observed in animals at 100 mg/kg/day of ICL 670 with standard diet (non-iron-supplemented) and included signs of decreased defecation and dehydration.
2. **Mortality:** One animal (100 mg/kg/day, standard diet) died during week 3.
3. **Body Weight:** The mean initial and final body weights of control (no iron-supplement) group were 180.8 and 336.3 g, respectively. The mean initial and final body weights of control (with iron supplemented-diet) were 180.2 and 344.2 g, respectively. The body weight of animals at 100 mg/kg (non-iron-supplement) was reduced (86% of control, control = 336.3 g) on day 29. The body weight of rats at 100 mg/kg/day (with iron-supplement) was 99% of control (control = 344.2 g) at the same time point.
4. **Food Consumption:** The mean initial and final food consumption of control (no iron-supplement) group were 21.6 and 23.2 g/day, respectively. The mean initial and final food consumption of control (with iron supplemented-diet) were 22 and 23.9 g/day, respectively. The food consumption of animals at 100 mg/kg (non-iron-supplement) was reduced (83% of control, control = 23.2 g/day) on day 29. The food consumption of rats at 100 mg/kg/day (with iron-supplement) was 95% of control (control = 23.9 g/day) at the same time point.
5. **Hematology:** No treatment-related changes were noted.
6. **Clinical Chemistry:** In animals on a normal diet and dosed with 100 mg/kg/day of ICL 670, very modest increase in urea (115% of control, control = 21.3 mg/dL), creatinine (132% of control, control = 0.31 mg/dL), and chloride (101% of control, control = 101 mEq/L) were noted. In animals on a normal diet at 100 mg/kg/day of ICL 670, serum iron concentration increased (212% of control, control = 243 µg/dL). In case of animals with iron-supplemented diet at 100 mg/kg/day, serum iron-concentration also increased to a lesser extent (138% of control, control = 225 µg/dL).
7. **Organ Weights:** Statistically significant organ weight changes were seen for animals on a standard diet at 100 mg/kg/day, which included increases in relative (to body weight) liver (109% of control, control = 2.749%), and kidney (126% of control, control = 0.584%) weights.
8. **Gross Pathology:** Treatment-related macroscopic findings in the animal that died early included multiple, dark foci in the nonglandular stomach, dark/red discoloration in the mucosa of the ileum, and distention of the urinary bladder with red/brown fluid-filled contents.
9. **Histopathology:** Treatment-related changes were observed in animals on a standard diet at 100 mg/kg/day of ICL 670 and included kidney (marked renal cortical tubular epithelial vacuolation in 3/10 animals and minimal to slight adrenal medullary vacuolation in 3/10 animals), gastrointestinal tract (gastric erosion in 2/10 animals), and thymus (thymic atrophy in 2/10 animals).

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10. **Toxicokinetics:** ICL 670 was given once daily for at least 4 weeks by gavage at a dose of 100 mg/kg. Blood samples were taken from the animals on Days 1 and Days 29 for the measurement of plasma concentrations of the drug and its iron complex. The toxicokinetic parameters of total ICL 670 in 4-week oral toxicity study in rats given normal or iron-supplemented rodent feed (from vol. 20, page 8-5169 of sponsor's submission):

Normal Feed					Iron-supplemented feed				
Dose/ mg/kg	Day	Cmax	Tmax	AUC	Dose/ mg/kg	Day	Cmax	Tmax	AUC
100	1	106	2	1030	100	1	103	2	409
100	29	61.1	6	846	100	29	107	2	460

Cmax (µg/ml); Tmax (hr); AUC (0-24hr) (µg.h/ml).

On day 1, the systemic exposure of rats given normal or iron-supplemented diet was found to be similar based on Cmax values. However, it was found to 2-2.5 times lower in animals given iron-supplemented diet because of the rapid clearance of the drug between 2 hour and 6 hour.

In a 4-week oral (gavage) exploratory study in male rats, animals (iron-supplemented and non-iron-supplemented diet) were treated with 100 mg/kg/day of ICL 670. No observed effect level was not established. Treatment-related microscopic findings were only limited to the animals fed on standard diet with treated with 100 mg/kg of ICL 670. Histopathological changes were seen (in those animals on standard diet and at 100 mg/kg/day of ICL 670) in the kidney (renal cortical tubular epithelial vacuolation in 3/10 animals), adrenal gland (medullary vacuolation in 3/10 animals), stomach (gastric erosion in 2/10 animals), and thymus (atrophy in 2/10 animals). ICL 670 was considered well tolerated in the animals fed an iron-supplemented diet at 100 mg/kg dose level, based on the absence of any toxicological findings. Based on the results of this study, the 30, 80, and 180 mg/kg doses were selected for the 26-week study in rats with iron-supplemented feed.

Rat

4-Week Oral Toxicity Study in Rats (Study No. 974080)

Testing Laboratory: Novartis Pharmaceuticals Corporation
Summit, NJ, USA

Date Started: August 25, 1997

Date Completed: October 8, 1997

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

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Animals: Wistar Hannover (HsdBrl:WH) rats were used throughout the study. Animals were approximately 7-8 weeks of age and the body weight ranges were 227.4 g-279.6 g for males and 163.4-202.4 g for females.

Drug Batch: ICL 670. Lot No. 800196.

Methods: ICL 670 was administered orally via gavage to rats (16/sex/group) at daily doses of 10, 30, and 100 mg/kg for at least 4 consecutive weeks at a dose volume of 10 ml/kg. The control group received vehicle [aqueous suspension of 0.5% Klucel (hydroxypropyl cellulose)] at an equivalent dose volume. The dose selection was made based on the results of the 2-week oral dose-range finding study in rats (study no. 974043). The detailed report of this study was not submitted in this submission; however, brief description of the study was provided which is described in the following paragraph.

In this range-finding study (study no. 974043), Wistar rats (5/sex/group) were treated with ICL 670 by oral gavage at doses of 200, 400, and 800 mg/kg for up to 10 days as the study was terminated on Day 11 due to excessive mortality at doses ≥ 400 mg/kg by Day 6. Mortality was observed at 200 (4/10), 400 (10/10), and 800 (8/10) doses between 2-10 days post dose. Histopathological changes were observed at 200 mg/kg in different organs that included cortical changes in the kidney (cortical tubular cytoplasmic vacuolation), organ hemorrhage, lesions in the stomach (dilatation, thinning of the wall, sporadic erosion and ulceration), decreased hematopoiesis in the spleen and splenic lymphoid depletion. As mortality was observed at 200 mg/kg dose level, 100 mg/kg was selected as the highest dose in the current study that will produce definitive toxicological effects and no mortality. The lowest dose of 10 mg/kg was chosen, which will show no effect and an intermediate dose of 30 mg/kg was expected to produce a dose-related effect.

In the current study, clinical signs /mortality was observed at least once daily during the predose, daily after dosing. Body weights and food consumptions were recorded on predose day 8 and weekly thereafter. Ophthalmoscopic examinations were performed on predose day 8 and on dosing day 29. Hematology, serum chemistry and urinalysis were performed on all surviving animals scheduled for terminal necropsy and on all surviving animals at the end of the recovery period. Organ weights were taken for adrenal glands, brain, heart, kidneys, liver, lung, ovaries, pituitary gland, prostate, spleen, testes, and thymus. Microscopic examinations were conducted on liver, kidney, heart, stomach, adrenal gland, etc.

Results:

1. **Clinical Signs:** Treatment-related clinical signs were observed in animals prior to death or sacrifice as well as in animals at 100 mg/kg that survived to study termination. These signs included ataxia, chromodacryorrhea, chromorhinorrhea, diarrhea, hunched posture, hypoactivity, hypothermia, labored breathing, perineal staining, skin loss, etc.
2. **Mortality:** There were ten deaths. Two deaths were reported to be incidental: one animal at 100 mg/kg died during orbital bleeding on Day 16 and one female at 10 mg/kg was sacrificed for humane reasons on Day 18. The remaining eight mortalities (five males and three females) occurred at 100 mg/kg and considered to be treatment-related.

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3. **Body Weight:** Treatment-related reductions in body weight were observed in both sexes at 100 mg/kg dose level. The initial (baseline) and final (week 5) body weights in the control male rats were 251 and 331 g, respectively. The initial and final body weights in the control females were 181 and 217 g, respectively. The body weight in males and females at 100 mg/kg during week 5 were 85% and 90% of the corresponding control values, respectively. No treatment-related change was noticed in either sex at doses ≤ 30 mg/kg.
4. **Food Consumption:** Treatment-related reductions in mean food consumption was noticed in both sexes at 100 mg/kg dose level. The initial (baseline) and final (Day 29) amounts of food consumed in the control males were 161 and 157 g, respectively. The initial and final food consumption values in the control females were 120 and 119 g, respectively. The food consumption in males and females at 100 mg/kg on Day 29 was found to be 76% and 92% of the corresponding control values, respectively. However, no treatment-related changes were noted in either sex at doses ≤ 30 mg/kg.
5. **Ophthalmology:** Ophthalmoscopic examinations did not reveal any ocular changes attributable to drug treatment.
6. **Hematology:** A significant increase in the red blood cell (RBC) distribution width was observed at 100 mg/kg in males (140% of control) and females (130% of control) compared to control (14.1 and 13.7 in males and females, respectively). Significant changes were observed in the hematology parameters in the females at 100 mg/kg dose. Hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were found to be about 91%, 91%, 92%, and 92%, respectively of control (Hb: 14.1 g/dL; Hct: 42.9%; MCV: 54.2 fl.; MCH: 19.6 pg) in the females at 100 mg/kg dose.
7. **Clinical Chemistry:** Significant changes in the serum chemistry parameters were observed in males at 100 mg/kg dose. Total bilirubin, AG ratio, glucose, cholesterol, unsaturated iron binding capacity, total iron binding capacity in males were reported to be 300%, 118%, 67%, 140%, 220%, and 159%, respectively of control values (total bilirubin: 0.1 mg/dL; AG ratio: 2.74; glucose: 141 mg/dL; cholesterol: 55 mg/dL; unsaturated iron-binding capacity: 300; total iron-binding capacity: 502). In females, AG ratio, creatinine, unsaturated iron binding capacity, iron, and total iron binding capacity were found to be 113%, 120%, 262%, 77%, and 143%, respectively of control values (AG ratio: 3.23; creatinine: 0.45 mg/ml; unsaturated iron binding capacity: 170; iron: 308; total iron binding capacity: 478) at 100 mg/kg dose.
8. **Urinalysis:** Urine pH was reported (from vol. 11, page 8-2020 of sponsor's submission) to be lowered at 100 mg/kg dose in both sexes (data missing from the submission, Table 3.3 only contains the urine specific gravity data and no information regarding urine pH was found in this table).
9. **Organ Weights:** Treatment-related changes in adrenal and thymus weights were observed in both sexes, which may have some correlation to histopathological changes observed in the respective organs.

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Adrenals: At the end of the treatment period, absolute weights of the adrenals for male rats at 100 mg/kg were decreased to 86% of the control (0.07 g). No change in relative spleen weight was noted in males at this dose level. At the end of the recovery period, the absolute adrenal weights for male rats returned to control values. In the females, absolute weights of the adrenals at 100 mg/kg were decreased to 87.5% of control (0.08 g), which returned back to control level at the end of the recovery period.

Kidney: Relative kidney weights for male rats at 30 and 100 mg/kg were increased to 107% and 132% of control (0.693%), respectively. At the end of the recovery period, the relative kidney weights for male rats at 100 mg/kg was increased to 113% of control (0.642%). No significant change in the absolute kidney weight was observed. In the females, the relative weights of the kidneys at 100 mg/kg increased to 117% of control (0.751%), which came back to normal at the end of the recovery period.

Liver: Relative weights of liver for male rats at 100 mg/kg were increased to 117% of control (2.914%). At the end of the recovery period, the relative weight of the liver for male rats still increased to 115% of control (2.666%). In the females, the relative weights of the livers at 100 mg/kg increased to 112% of control (3.053%), which returned back to control level at the end of the recovery period.

Testes: Relative weights of testes were increased to 118% of control (1.08%) at 100 mg/kg dose and similar increase (118% of control: 0.915%) was also observed at the end of the recovery period.

Ovaries: The relative weights of the ovaries at 100 mg/kg were increased to 116% of control (0.045%), which came back to normal control level at the end of the recovery period.

Thymus: Absolute weights of the thymus were for male rats at 100 mg/kg were decreased to 54.5% of control (0.44 g). Relative weights of the thymus for male at 100 mg/kg were decreased to 64% of the control (0.145%). However, at the end of the recovery period the relative weights of the thymus for males at 100 mg/kg were increased to 154% of control (0.085%). In the females, the absolute thymus weights at 30 and 100 mg/kg were decreased to 83% and 73% of the control (0.41 g), respectively. Similarly, the relative thymus weights for females at 30 and 100 mg/kg were decreased to 86% and 82% of control (0.199%), respectively.

10. **Gross Pathology:** Treatment-related gross changes included red lesions in the stomach, red content in the intestine, urinary bladder and red mesenteric lymph node at 100 mg/kg doses in both sexes.

11. **Histonathology:** Treatment-related microscopic changes were observed generally at 100 mg/kg dose (unless otherwise mentioned) in the kidney (renal tubular vacuolation and tubular necrosis in both sexes), adrenal gland (vacuolation of adrenal medulla in both sexes), heart (myocardial degeneration in males at 100 mg/kg and in one female at 10 mg/kg and in another male at 30 mg/kg; granulation tissue in one male; inflammation in both sexes; chronic myocarditis in males), stomach (erosion in both sexes; hemorrhage and necrosis in males), large intestine (edema, erosion, hemorrhage, and inflammation in both sexes), small intestine (hemorrhage in males; epithelial hyperplasia in both sexes), liver (hepatocellular

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hypertrophy and single cell necrosis in males); pancreas (atrophy of acinar cells in males and females; edema in males), lymph node (erythrophagocytosis, histiocytic infiltration in both sexes at 100 mg/kg; lymphoid depletion in males; lymphoid hyperplasia in both sexes), spleen (atrophy and lymphoid depletion in both sexes), thymus (atrophy, hemorrhage, and necrosis in both sexes) and lung (hemorrhage and inflammation in both sexes).

Histopathological findings in rats treated with ICL 670 by oral gavage at doses of 10, 30, and 100 mg/kg for 4-weeks are shown in the following table (n = 10 at 0, 10, and 30 mg/kg and n = 12 at 100 mg/kg):

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Organ/Tissue	0		10		30		100	
	Male	Female	Male	Female	Male	Female	Male	Female
Heart	0	0	0	1	1	0	4	0
- Myocardial degeneration								
- Granulation tissue	0		0		0		1	
- Inflammation	1	0	0	0	0	0	1	1
- Chronic myocarditis	0		0		0		2	
Large Intestine								
- Edema	0	0	0	0	0	0	4	1
- Erosion	0	0	0	0	0	0	3	1
- Hemorrhage	0	0	0	0	0	0	6	2
- Inflammation								
Small Intestine								
- Hemorrhage	0	0	0	0	0	0	5	0
- Epithelial hyperplasia	0	0	0	0	0	0	3	1
Liver								
- Hepatocellular hypertrophy	0	0	0	0	0	0	2	0
- Single cell necrosis	0	0	0	0	0	0	2	0
Stomach								
- Erosion	0	0	0	0	0	0	4	1
- Hemorrhage	0	0	0	0	0	0	6	0
- Necrosis	0	0	0	0	0	0	4	0
Pancreas								
- Atrophy, acinar	0	0	0	0	0	0	1	1
- Edema	0	0	0	0	0	0	3	0
Kidney								
- Tubular vacuolation	0	0	0	0	0	0	9	4
- Tubular necrosis	0	0	0	0	0	0	7	3
Adrenal Gland								
- vacuolation, medulla	0	0	0	0	0	0	7	3
Lymph Node								
- Erythrophagocytosis	0	0	0	0	0	0	6	2
- Histiocytic infiltration	0	0	0	0	0	0	1	1
- Lymphoid depletion	0	0	0	0	0	0	3	0
- Lymphoid hyperplasia	0	0	0	0	0	0	3	2
Spleen								
- Atrophy	0	0	0	0	0	0	3	1
- Lymphoid depletion	0	0	0	0	0	0	5	3
Thymus								
- Atrophy	0	0	0	0	0	0	3	1
- Lymphoid depletion	0	0	0	0	0	0	2	2
- Hemorrhage	0	0	0	0	0	0	2	1
- Necrosis	0	0	0	0	0	0	4	2
Lung								
- Hemorrhage	0	0	0	0	0	0	3	1/11
- Inflammation	0	0	0	0	0	0	4	4/11

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Treatment-related findings in 4-week recovery period were limited to heart (fibrosis in 2/6 males at 30 mg/kg; leukocytic infiltration in 1/6 males at 30 mg/kg; aortic inflammation in 1/6 males at 30 mg/kg) and lymph node (plasmacytic hyperplasia in 1/6 females at 30 mg/kg and 1/6 males at 10 mg/kg).

12. **Toxicokinetics:** Male and female rats (no. of animals not mentioned) were administered with ICL 670 by oral gavage at doses of 10, 30, and 100 mg/kg once daily for 4 weeks. Blood samples were collected from the rats on Days 2 and Days 16. The concentrations of the drug and its iron complex were measured by HPLC technique.

The apparent pharmacokinetic parameters of total ICL 670 (free + iron complex) in a 4-week oral toxicity study are summarized below (reproduced from vol. 19, page 8-5004 of sponsor's submission):

Dose	Day	Male Rats			Female Rats		
		Cmax	Tmax	AUC	Cmax	Tmax	AUC
10	2	2.40	0.5	9.64	4.90	0.5	10.3
10	16	2.90	0.5	11.6	7.76	0.5	15.4
30	2	13.5	2	68.3	21.2	2	97.2
30	16	14.5	2	72.2	16.2	2	69.7
100	2	91.2	2	826	103	2	747
100	16	112	2	850	127	2	904

Cmax (µg/ml): highest observed plasma concentration.

Tmax (hr): time to reach Cmax.

AUC (0-24hr) (µg.h/ml): area under the plasma concentration-time curve from t = 0 to 24 hour by the trapezoidal rule.

The absorption of ICL 670 was rapid after 10 mg/kg dose as Cmax reached as soon as 0.5 hr after treatment. The absorption was somewhat delayed at medium and high dose levels. The exposure increased overproportionately (60-90 times higher) from 10 to 100 mg/kg dose. No marked difference was observed in systemic exposure between males and females (except at 10 mg/kg dose level as Cmax values were 2 times higher in females than that of males).

In a 4-week oral (gavage) toxicity study in rats, animals received ICL 670 at 10, 30, and 100 mg/kg/day. Six rats/sex/group entered a 4-week recovery period following treatment. No observed adverse effect level (NOAEL) was established as 10 mg/kg. Mortality was seen at 100 mg/kg. The target organs of toxicity were kidney (renal tubular vacuolation, tubular necrosis at 100 mg/kg), adrenal gland (vacuolation of adrenal medulla at 100 mg/kg), heart (myocardial degeneration at 10 (1/10 female), 30 (1/10 male) and 100 mg/kg), stomach (erosion, hemorrhage, necrosis at 100 mg/kg), large intestine (edema, erosion, hemorrhage, inflammation at 100 mg/kg), large intestine (edema, erosion, hemorrhage, inflammation at 100 mg/kg), small intestine (hemorrhage, epithelial hyperplasia at 100 mg/kg), liver (hepatocellular hypertrophy, single cell necrosis at 100 mg/kg), pancreas (atrophy of acinar cells, edema at 100 mg/kg), lymph node (erythrophagocytosis, histiocytic infiltration, lymphoid depletion, lymphoid hyperplasia at

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100 mg/kg), spleen (atrophy, lymphoid depletion at 100 mg/kg), thymus (atrophy, lymphoid depletion, hemorrhage, necrosis at 100 mg/kg) and lung (hemorrhage, inflammation at 100 mg/kg). Following a 4-week recovery period, histopathological changes were noted in the heart and lymph node were still evident at 30 mg/kg (heart and lymph node) and 10 mg/kg (lymph node).

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26-week Oral (Gavage) Toxicity Study in Rats
(987037)

Testing Laboratory: Novartis Pharmaceutical Corporation
East Hanover, NJ

Study Start and Completion Dates: November 2, 1998 and
November 16, 1999

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Male (218.7-305.2 g, 9 weeks old)
Female (159.8-216.3 g, 9 weeks old)
Wistar Hannover(HsdBrl:WH) rats

Methods: To determine the potential toxicity and to select adequate doses for the 2-year carcinogenicity study in rats, ICL670 was given by oral gavage to rats (20/sex/group) at 30, 80, and 180 mg/kg/day for 26 weeks. There were two control groups (control1 received iron supplemented diet and control2 received standard diet). Animals in the treatment groups also received iron supplement diet (~1680 ppm). The dose selection was based on the results of a 4-week oral (gavage) toxicity study in rats. In that study, rats were treated with ICL670 by oral gavage at 0, 10, 30, and 100 mg/kg/day for 4 weeks. The high dose was lethal and the low dose was no observed adverse effect dose. ICL670 was also lethal at 100 mg/kg/day in another 4-week exploratory study in rats. It appears that the high dose selected (180 mg/kg/day) in the 26-week oral toxicity study in rats is excessive. In the 26-week oral toxicity study, clinical sign of toxicity and mortality were observed daily. Body weights and food consumption were determined weekly. Ophthalmology examination was conducted before treatment, and during weeks 4, 13, 26, and recovery week 4. Hematology and clinical chemistry were determined during weeks 4, 13, 26, and recovery period. All animals were necropsied at termination. All animals from all groups were examined histopathologically. Organ weights were determined at necropsy. Plasma levels of the test drug were determined on days 1 and 2 and during weeks 12/13 and 25/26 using HPLC.

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Results:

1. Clinical Signs: The treatment related clinical signs of toxicity were found mainly in the mid and high dose groups. These included ocular opacities (6 of 20 males and 3 of 20 females in mid dose group and 27 of 30 each sex in the high dose group) and exophthalmos (1, 0, 2, 1, and 3 for control1, control2, low, mid, and high dose males or 0, 0, 1, 4, and 9 for control1, control2, low, mid, and high dose females, respectively).

2. Mortality: The high dose was lethal (10 of 30 males and 2 of 30 females were found dead or sacrificed). In most cases, the cause of death was attributed to renal toxicity. There were 7 animals in the control and treatment groups either sacrificed or found dead due to gavage error. Due to excessive mortality, the high dose males were terminated at week 19.

3. Body Weight: The mean initial and final body weights in the control group (standard diet, control2) were 260 and 498.8 g for males or 183.8 and 279.2 g for females. The body weight information is summarized in the following table.

Absolute body weights and body weight gain in parenthesis in males

Treatment days	Control1	Control2	Low dose	Mid dose	High dose
Day 1	259.2	260.0	263.4	260.3	259.0
Day 29	348.2	351.7	359.2	351.6	344.8
Day 92	425.0	439.1	443.8	429.0	396.7
Day 134	461.0 (201.8)	472.9 (212.9)	487.6 (224.2)	464.6 (204.6)	412.6 (153.6)
Day 183	478.6 (219.4)	498.8 (239.8)	514.5 (251.1)	492.4 (232.1)	-----

Absolute body weights and body weight gain in parenthesis in females

Treatment days	Control1	Control2	Low dose	Mid dose	High dose
Day 1	182.0	183.8	183.2	180.7	181.6
Day 29	221.3	223.2	222.6	219.3	218.5
Day 92	250.2	254.2	248.3	249.1	246.1
Day 134	270.6	270.8	267.1	271.5	262.2
Day 183	281.7 (99.7)	279.2 (95.4)	280.0 (96.8)	289.8 (109.1)	279.9 (98.3)

The terminal body weight gain (on treatment day 134) was decreased by ~28% in the high dose males as compared to the control. The terminal body weight gain was not changed in the treated females as compared to the control.

4. Food Consumption: The average food consumption in the control group (standard diet) was 23.3-25.8 g/animal/day in males or 16.7-19.1 g/animal/day for females. The average food consumption was 22.6-27.1, 22.1-26.4, and 18.8-26.2 g/animal/day

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in the low, mid, and high dose males or 16.5-19.2, 16.6-19.2, and 17.3-19.5 g/animal/day in the low, mid, and high dose females, respectively. The food consumption was significantly decreased on day 134 in the high dose males as compared to the control.

5. Ophthalmology: There was treatment related increase in the incidence of cortical lenticular aberrations (cataracts) in treated males and females. The aberrations were characterized as posterior polar vacuoles, anterior/posterior cortical striations or mature cataracts. Most of these changes were bilateral. The incidence of these changes were summarized in table 4.2-1 on page 28 of this report. This table is attached below.

Table 4.2-1. Summary of compound-related lenticular aberrations by interval

Week 4					
Group	1	2	3	4	5
Males	0/30	0/29	0/20	0/20	0/30
Females	0/30	0/30	0/20	0/20	0/30
Week 11					
Group	1	2	3	4	5
Males	0/28	—	—	4/19	13/27
Females	0/30	—	—	1/20	2/30
Week 13					
Group	1	2	3	4	5
Males	0/28	0/29	0/20	5/19	26/27
Females	0/30	0/30	0/20	0/20	7/30
Week 18 (term evaluation for males Groups 1, 2, and 5 prior to early sacrifice)					
Group	1	2	3	4	5
Males	0/27	0/29	—	—	20/20
Week 26 (term)					
Group	1	2	3	4	5
Males	0/19	0/19	2/20	5/19	—
Females	0/30	0/30	0/19	2/20	28/28
Recovery males (week 23)					
Group	1	2	3	4	5
Males	0/30	0/5	—	—	6/6
Recovery females (week 31)					
Group	1	2	3	4	5
Females	0/10	0/10	—	—	8/10

Note: Dose levels: Groups 1-5 correspond to 0 (supplemented with iron), 0 (unsupplemented with iron), and 30, 80, and 180 mg/kg/day, respectively.

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6. Hematology: Treatment significantly increased red blood cell count, hematocrit, and hemoglobin and decreased mean corpuscular hemoglobin and mean red cell volume in mid and high dose males. In females, however, the treatment significantly decreased hematocrit, hemoglobin, mean corpuscular hemoglobin, and mean red cell volume. The red blood cell distribution width, nucleated red blood cell, and reticulocyte were significantly increased in mid and high dose males and females. Slight increase in the platelet count was noted in the high dose males and females. The results are summarized in the following table.

Hematology parameters measured at termination in males

	Control1	Control2	Mid dose	High dose
RBC	8.63	8.94	9.83	10.56
Hematocrit (%)	44.9	45.9	48.8	51.4
Hemoglobin (g/dl)	15.4	15.7	16.5	17.3
Mean corpuscular hemoglobin (pg)	17.9	17.5	16.8	16.4
Mean red cell volume (fl)	52.0	51.5	49.7	48.9
RBC Dist. Width (%)	15.1	15.4	20.3	26.9
Nucleated RBC	0	0	0.4	24
Reticulocytes (%)	2.5	2.9	3.3	6.0
Platelet (k/l)	967.7	969.1	979.3	1013

Hematology parameters measured at termination in females

	Control1	Control2	Mid dose	High dose
RBC	7.96	7.95	8.02	7.62
Hematocrit (%)	43.4	43.4	39.9	38.0
Hemoglobin (g/dl)	15.0	14.9	13.5	13.0
Mean corpuscular hemoglobin (pg)	18.9	18.8	16.9	17.1
Mean red cell volume (fl)	54.6	54.6	49.8	49.9
RBC Dist. Width (%)	13.9	14.2	19.2	20.9
Nucleated RBC	0	0	0.7	3
Reticulocytes (%)	2.9	2.3	4.2	7.8
Platelet (k/l)	879	873	940	982

7. Clinical Chemistry: The most notable change was increase in blood urea nitrogen (8% in mid dose males and 27-29% in high dose males) and creatinine (12-23% in mid dose males and 36-37.5% in high dose males). This change was not seen in females.

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8. Urinalysis: Increased acidity of urine was noted in a few males treated with the test drug at 80 mg/kg/day or higher.

9. Organ Weights: Spleen weight was significantly increased in the high dose males (103%) and females (50%) as compared to the control. The kidney weight was significantly increased in the high dose males (22%) and females (14%) as compared to the control. The kidney weight in the low and mid dose males was also increased by 18% and 19%, respectively.

10. Gross Pathology: Treatment related ocular opacity was observed in 4 of 20 males in the mid dose group and 17 of 24 males and 15 of 20 females in the high dose group. Splenic enlargement was noted in 7 of 20 males in the high dose group. Discoloration of the stomach was observed in 6 of 24 high dose males.

11. Microscopic Pathology: The treatment related changes were observed in the eye, kidney, adrenal gland, spleen, and stomach. In eyes, the changes (cataracts) were characterized by lenticular degeneration and fragmentation, vacuole formation and/or lenticular epithelial hyperplasia. The incidence of cataracts was 21 of 24 males and 16 of 19 females in the high dose group and 2 of 20 males in the mid dose group. In the high dose group, cytoplasmic vacuolation of cortical tubular epithelium of kidney in 9 of 24 males and 2 of 20 females and cytoplasmic vacuolation of adrenal medulla in 7 of 24 males. Increased splenic hematopoiesis was observed in 4 of 24 males and 2 of 20 females in the high dose group. Ulceration/erosion of the glandular stomach was noted in 1 of 20 mid dose and 5 of 24 high dose males. These are summarized in the following table.

Incidence of histopathological findings

	Control1	Control2	Low dose	Mid dose	High dose
Eye:					
Cataracts				2m	21m 16f
Kidney:					
Cytoplasmic vacuolation of cortical tubular epithelium					9m 2f
Adrenal					
Cytoplasmic vacuolation of adrenal medulla					7m
Spleen:					
Increased splenic hematopoiesis					4m 2f
Stomach:					
Ulceration/erosion of glandular stomach				1m	5m

m = males, f = Females

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12. Plasma Levels: The maximum plasma level of test drug was detected within 2 hours in most cases. The plasma level of test drug was reached steady state at week 12. No evidence of drug accumulation was observed over time as compared the plasma levels of the test drug during weeks 12 and 25. The data were summarized in Table 4.5-1 on page 33 in this report. This table is attached below.

Table 4.5-1. Summary of toxicokinetics of total ICL670 in the rats

Dose (mg/kg)	Parameter	Males			Females		
		Day 1	Week 12	Week 25	Day 1	Week 12	Week 25
30	C _{max} (µg/mL)	7.29	31.7	25.5	27.0	24.1	7.49
	t _{max} (h)	2	2	2	2	0.5	0.5
	AUC(0-24h) (µg.h/mL)	36.6	95.7	61.4	61.9	59.3	20.8
80	C _{max} (µg/mL)	39.7	101	134	84.6	105	52.4
	t _{max} (h)	2	2	2	2	2	2
	AUC(0-24h) (µg.h/mL)	101	431	458	287	314	167
180	C _{max} (µg/mL)	132	202	-	118	215	131
	t _{max} (h)	2	2	-	4	2	4
	AUC(0-24h) (µg.h/mL)	765	1270	-	958	1350	1350

*Samples were not obtained at the week 25 interval due to the early sacrifice of these animals.

Sponsor stated that a clinically effective dose is expected between 5 and 40 mg/kg. The AUC_{0-24hr} of ICL670 in humans following a single oral dose of 20 mg/kg was 388 µmol.h/l or 144.5 µg.h/ml. The AUC_{0-24hr} of ICL670 in rats following oral dose of 80 mg/kg/day (431 µg.h/ml in males or 314 µg.h/ml in female at week 12) is approximately 2-3 folds of that in humans after 20 mg/kg.

In summary, ICL670 was given by oral gavage to rats at 30, 80, and 180 mg/kg/day for 26 weeks. High dose of 180 mg/kg/day was lethal and exceeded MTD. ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts)

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and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, MTD was identified between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to MTD in females.

Study Title: 2-Week Intravenous Toxicity Study in the Rat

Key Study Findings: In this study, animals were tested intravenously at 13.5, 27 and 54 mg/kg/day for 14 days. The maximum tolerated dose (MTD) appeared to be 27 mg/kg/day. A no observed effect level (NOEL) could be determined, as renal toxicity was observed at all tested dose levels. The target organ of toxicity appeared to be the kidney (tubulonephrosis, tubular basophilia/regeneration).

Report No/Study No: 0310064

Volume #, and Page #: Vol. 1 of 2, page # 1

Conducting Laboratory and Location: Novartis Pharma AG, Basel, Switzerland.

Date of Study Initiation: October 6, 2003

Date of Study Completion: December 5, 2003

GLP Compliance: This is a draft report and the GLP compliance statement was not signed.

QA Report: yes () no (X)

Drug, Lot #, and % Purity: ICL-670, Batch No. 4469, ζ η

Formulation/Vehicle: Solution/Polyoxyl 35 castor oil (Cremophor EL, 65% w/v) and ethanol.

Methods: The purpose of this study was to determine the toxicity of ICL670 in rats after prolonged i.v. infusion and to support the proposed bioequivalence (BE) study in humans. ICL670, supplied as a solution (90 mg/5 mL) in Cremophor EL (65% w/v) and

ethanol in ampoules, was diluted with 5% glucose solution and was administered to rats (n = 5/sex/group) as a continuous i.v. infusion (2.5 mL/kg/hr) at 0, 13.5, 27 and 54 mg/kg/day for 2 weeks. Vehicle control animals received the reference item (placebo for ICL670: a solution of Cremophor EL and ethanol that was diluted with 5% glucose solution). The following examinations were performed: mortality, clinical signs, body weight, food consumption, clinical pathology, organ weights, gross and microscopic examinations and toxicokinetic measurements.

Dosing:

Species/Strain: Rats – WI \ – (BRL/HAN) IGS BR

No./Sex/Group (Main Study): 5/sex/group

Satellite Groups used for Toxicokinetics or Recovery: None

Age: 12 – 16 weeks

Weight: 190 to 335 g

Doses in Administered Units: 0, 13.5, 27 and 54 mg/kg/day.

The study design is shown in the following Table (Table 3-2 from Vol. 1, page 18 of sponsor's submission):

Study Design

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Table 3-2 Study design, animal allocation and test item dosages

	Group 1		Group 2		Group 3		Group 4	
	Reference Item		ICL670		ICL670		ICL670	
	Male	Female	Male	Female	Male	Female	Male	Female
Dosage (mg/kg/day)*		0		13.5		27		54
Dosage volume (mL/kg)		2.5		2.5		2.5		2.5
Number of animals	5	5	5	5	5	5	5	5
Animal nos.	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40
Cage nos.	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40

* Expressed in terms of the base.

Reserve animals (group 5): male no. 41-48 (cage no. 41-48), female no. 49-66 (cage no. 49-66).

Basis of Dose Selection: The doses were selected based on the results of a previous acute i.v. toxicity study in rats. In that study, a single i.v. administration up to 50 mg/kg was well tolerated. At 75 mg/kg, discolored urine was observed.

Due to limited solubility of the test drug and solvent-related toxicity a maximal dose of 54 mg/kg/day was chosen as the high dose in the current study.

Route, Form, and Dose Volume: intravenous, solution, 2.5 mL/kg

Observations and times:

Clinical Signs: Clinical signs were observed once daily during the pretest, at least twice daily during the treatment period.

Body Weights: Body weights were recorded once during pretest, twice weekly during the treatment period.

Food Consumption: Food consumption was recorded once during pretest, twice weekly during the treatment period.

Hematology: Blood samples were collected for hematology at necropsy.

Clinical Chemistry: Blood samples were collected for serum chemistry at necropsy.

Ophthalmoscopy: None

Gross Pathology: Gross pathology was conducted at necropsy.

Organs Weighed: The following organs were weighed from all main study animals: adrenals, aorta, bone marrow (in bone), brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerves, Harderian glands, heart, ileum, jejunum, kidneys, knee joint, lacrimal glands, larynx, liver, lung, lymph node (mandibular, mesenteric), mammary area, nasal cavity, ovaries, pancreas, peripheral nerves, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, uterus, urinary bladder and vagina.

Histopathology: The following organs/tissues were examined for histopathology from control and high dose animals: adrenals, aorta, bone marrow (in bone), brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerves, Harderian glands, heart, ileum, jejunum, kidneys, knee joint, lacrimal glands, larynx, liver, lung, lymph node (mandibular, mesenteric), mammary area, nasal cavity, ovaries, pancreas, peripheral nerves, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, uterus, urinary bladder and vagina. In addition, kidneys, liver, mesenteric lymph node, pancreas, spleen, thymus, uterus, urinary bladder and vena cava abdominals from all animals of other dose groups were processed histologically and examined microscopically.

Toxicokinetics: Blood samples were collected from all main group animals at the end of the treatment period. The samples were analyzed by $\square$

Results:

Mortality: There was no mortality.

Clinical Signs: No test-article-related clinical signs were observed at any tested doses.

Body Weights: The mean initial and final body weight of control males were 227 and 302 g, respectively. The mean initial and final body weight of control females were 207 and 225 g, respectively. In males, mean body weight gain was significantly increased (128% and 116% of control at 13.5 and 27 mg/kg/day, respectively, control weight gain = 25 g) at 13.5 and 27 mg/kg/day compared to control. However, at 54 mg/kg/day, mean body weight gain was significantly reduced (52% reduction in body weight gain) in males compared to control. In females, mean body weight gain was reduced at all doses (16.7%, 11.2% and 38.9% reduction in body weight gain) compared to control weight gain (18 g). The following table shows the body weight data in males and females after treatment with ICL670 in rats.

Mean body weight data in males and females

Body weight (g) of males

Treatment (mg/kg/day)	Initial	Week 2	% of Control (Week 2)	Weight Gain (Final-Initial) (g)	Weight Gain % of Control (Week 2)
0	277	302	100.0	25	100
13.5	296	328	108.6	32	128.0
27	279	308	101.9	29	116.0
54	309	321	103.9	12	48.0

Body weight (g) of females

Treatment (mg/kg/day)	Initial	Week 2	% of Control (Week 2)	Weight Gain (Final- Initial) (g)	Weight Gain % of control (Week 4)
0	207	225	100.0	18	100
13.5	203	218	96.8	15	83.3
27	215	231	102.6	16	88.8
54	212	223	99.1	11	61.1

Food Consumption: The mean initial and final food consumption in control males were 20.1 and 17.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 16.4 and 16.0 g/animal/day, respectively. The final food consumption in males at 54 mg/kg/day was reduced by 15% when compared to control.

Hematology: No significant treatment-related changes were observed at any of the tested doses.

Clinical Chemistry: Serum magnesium concentration was increased in a dose-related manner (males: 13%, 13% and 15% increase at low-, mid- and high-dose, respectively, over control, control = 1.05 mM; females: 15% increase at 27 mg/kg/day compared to control, control = 1.09 mM). Although not significant, creatinine concentration was increased in both sexes at high dose (6-12% in males, control = 49 μ M; females: 4-19% in females, control = 52 μ M).

Ophthalmoscopy: None.

Organ Weights: Kidney weights (absolute: males: 115% of control at 54 mg/kg/day, control = 2.33 g; females: 113% of control at 54 mg/kg/day, control = 1.67 g) were increased in both sexes at high dose. However, these changes were not statistically significant.

Gross Pathology: The following changes were observed: enlarged adrenal gland in 2 of 5 females at high dose. However, this macroscopic finding had no evident microscopic correlate and was considered to be of no toxicological significance.

Histopathology: Treatment-related histopathological changes were observed in the kidney (tubulonephrosis, tubular basophilia/regeneration) at all dose levels in both sexes. The following table (Table 4-1 from Vol. 1, page 26 of sponsor's submission) shows relevant treatment-related microscopic changes.

Treatment-related relevant microscopic findings

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Table 4-1 Relevant microscopic findings related to test item

Dosage (mg/kg/day) Number of Animals	Males				Females			
	0	13.5	27	54	0	13.5	27	54
	5	5	5	5	5	5	5	5
Kidneys:								
Tubulonephrosis	5	5	5	5	5	5		
Total Affected	-	4	5	5	5	5		5
Mean Severity	-	1.3	2.2	2.8	1.2	1.4		2.6
Tubular basophilia/regeneration								
Total Affected :	-	-	2	5	4	4		5
Mean Severity :	-	-	1.0	1.2	1.8	2.0		2.4

Codes and symbols used at finding level

GRADE 1	=	minimal	/ very few	/ very small
GRADE 2	=	slight	/ few	/ small
GRADE 3	=	moderate	/ moderate number	/ moderate size
GRADE 4	=	marked	/ many	/ large
GRADE 5	=	massive	/ extensive number	/ extensive size

Vehicle-related changes were observed in the kidney (proximal tubule vacuolation), spleen (histiocytosis with vacuolation, red pulp), mesenteric lymph node (histiocytosis with vacuolation, sinus) and vena cava (thrombus, intimal proliferation and vasculitis/perivasculitis). The following table (Table 4-2 from Vol. 1, page 26-27 of sponsor's submission) shows relevant microscopic findings that are not apparently related to test article.

Relevant microscopic findings that are possibly related to the vehicle

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Table 4-2 Relevant microscopic findings related to vehicle or mode of administration

Dosage (mg/kg/day) Number of Animals	Males				Females			
	0	13.5	27	54	0	13.5	27	54
	5	5	5	5	5	5	5	5
Kidneys: Vacuolation, proximal tubules	5	5	5	5	5	5		
Total Affected	5	2	4	5	4		5	5
Mean Severity	1.8	1.0	1.3	2.0	1.8		1.0	2.4
Spleen: Histiocytosis with vacuolation, red pulp	5	5	5	5	5			
Total Affected :	5	-	-	5	5			5
Mean Severity :	3.4	-	-	3.0	3.0			2.6
Mesenteric lymph node: Histiocytosis with vacuolation, sinus	5	5	5	5	5	5		
Total Affected :	5	-	-	5	5			5
Mean Severity :	2.8	-	-	2.4	2.6			2.4

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Dosage (mg/kg/day) Number of Animals :	Males				Females			
	0	13.5	27	54	0	13.5	27	54
	5	5	5	5	5	5	5	5
Vena cava: Thrombus	4	5	5	4	5	5	5	4
Total Affected :	1	1	3	3	4	5	5	3
Intimal proliferation								
Total Affected :	4	5	4	4	5	5	5	4
Mean Severity :	2.0	2.4	2.5	2.0	2.0	2.0	1.6	2.0
Vasculitis/perivasculitis								
Total Affected :	1	3	3	3	3	4	3	2
Mean Severity :	1.0	1.0	1.3	1.0	1.0	1.3	1.3	1.5

Codes and symbols used at finding level as for table 4-1

Toxicokinetics: Plasma concentration profile at the end of the 14-day infusion period reflected a steady state concentration. Plasma concentrations increased over proportionally in both sexes. Mean concentrations were similar in males and females at 13.5 mg/kg/day; however, a sex-related difference in plasma concentration was seen at 27 and 54 g/kg/day. Males showed slightly higher drug levels than females. The following table (Table 4-3 from Vol. 1, page 27 of

sponsor's submission) shows the mean concentration of total ICL670 in rat plasma at the end of the infusion period.

Mean concentration of total ICL670 in rat plasma at the end of the infusion period

Table 4-3 Mean concentration of total ICL670 in rat plasma at the end of the infusion period

Dose Parameter	mg/kg/day Units	Group 2 13.5		Group 3 27		Group 4 54	
		Female	Male	Female	Male	Female	Male
C	µg/mL	0.676	0.673	1.10	1.77	4.31	7.83
C/Dose	(µg/mL)/(mg/kg/day)	0.0501	0.0499	0.0407	0.0656	0.0799	0.145

Summary: In this 2-week i.v. infusion toxicity study in rats, animals were tested intravenously at 13.5, 27 and 54 mg/kg/day for 14 days. The MTD appeared to be 27 mg/kg/day based on 10% reduction in body weight gain in males and close to 10% reduction in females relative to control. A NOAEL could not be determined, as renal toxicity was observed at all tested dose levels. The major target organ of toxicity appeared to be the kidney (tubulonephrosis, tubular basophilia/regeneration, etc.).

An oral neonatal and juvenile development dose ranging-finding study in rats (study #0370003)

Methods: To determine the adequate doses of ICL670 for a subsequent definitive neonatal and juvenile developmental study in rats, ICL670 was given orally by gavage to rats (12/sex/group) at 0, 30, 60, and 90 mg/kg/day (with and without iron supplement, except for group of 30 mg/kg/day with iron supplement only). The dosing was initiated on post partum day 7 and continued through day 34 post partum for a total of 28 days. The study design was summarized in Table 3-1 on page 15 in Amendment #055. This table is attached below.

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Table 3-1 Study design, animal allocation and test article doses

Group	Iron supplement	Number/group		Animal numbers ^a		Dose (mg/kg/day)	Concentration (mg/mL)
		male	female	male	female		
1	+	12	12	2-24	1-23	0	0
Control ^b							
2	-	11	13 ^b	34-54	33-57	0	0
Control							
3	+	12	12	66-88	65-87	30	15
Low ^p							
4	+	12	12	98-120	97-119	60	30
Mid ^p							
5	-	12	12	130-152	129-151	60	30
Mid							
6	+	12	12	162-184	161-183	90	45
High ^p							
7	-	12	12	194-216	193-215	90	45
High							

^aPSA computer assigned odd numbers to females and even numbers to males. Two studies were set up on the PSA system. Study 0370003M was used for processing males and study 0370003F was used to process females.

^bOne group 2 litter (Foster Dam No. 518) had 3 males and 5 females.

Mortality and clinical signs of toxicity were observed daily. Body weight was determined on post partum days 0, 7, 14, and 21. All treated animals were sacrificed on post partum day 35 and gross examination was conducted at necropsy.

Results: ICL670 was lethal to rats at doses of 60 mg/kg/day or higher. The mid and high dose groups were terminated on post partum day 14 or 16 due to high pup mortality. In the mid dose groups, 12 pups (10 females and 2 males) without iron supplement and 5 pups with iron supplement were found dead. In the high dose groups, 10 pups (8 females and 2 males) without iron supplement and 5 pups (2 females and 3 males) with iron supplement were found dead. Following clinical signs of toxicity were noted in these groups: decreased locomotor activity, recumbency, cold to touch, and pale appearance. Gross examination revealed discolored lungs, distended stomach, and blockage of the large intestine with hardened fecal material. All animals in the low dose group were survived until

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termination. There were no treatment related changes in this group.

In conclusion, ICL670 was lethal to rats at doses of 60 mg/kg/day. The dose of 30 mg/kg/day was no effect dose.

Study Title: An Oral Neonatal and Juvenile Development Study in Rats

Key Study Findings: In an oral toxicity study in juvenile rats (iron-supplemented), animals were treated with ICL670 at 0, 10, 20 and 40→30 mg/kg/day from post partum day 7 through 70. Mortality was observed at 40 mg/kg/day irrespective of iron supplementation. The target organ of toxicity appeared to be the eye (cataract) and kidney (inflammatory and degenerative changes consisting of tubular vacuolation and/or necrosis and tubular dilation and/or basophilia at 40→30 mg/kg/day in males and females). The NOAEL appeared to be 20 mg/kg/day in males and 10 mg/kg/day in females.

Study No.: 0370030

Volume # Page #: EDR/NDA21882/pharmatox/tox/0370030

Conducting Laboratory and Location: Novartis Pharmaceutical Corporation, East Hanover, NJ

Date of Study Initiation: October 7, 2003

Date of Study Completion: February 18, 2005

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, []

Methods: In this study, timed-pregnant female Wistar Hannover rats were used for pups. On the day of delivery, the pups were cross fostered to have litters of 5 pups per sex. Prior to the initiation of treatment post partum (pp) Day 7, litters were culled to 4 pups per sex and litters were assigned to Single-dose, Pathology and Main study groups as well as dose groups. Single-dose animals (N= 8/sex control or 16/sex/group) were used for toxicokinetics (TK) analysis after receiving one dose on Day 7 pp. Treatment was initiated on Day 7 pp for the Pathology (N= 12/sex/group) and Main study animals (N= 40/sex/group) and continued through at least Day 70 pp .

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ICL670 was administered orally by gavage as suspensions in 0.5% (w/v) hydroxypropylcellulose (Klucel, aqueous solution) to four groups of pups (40 pups/sex/group for Main study groups and 12 pups/sex/group for Pathology groups) at doses of 10 (with iron supplemented feed), 20 (with iron supplemented feed) and 40 mg/kg/day (with and without iron supplemented feed). An additional 2 groups (40 pups/sex main study or 12 pups/sex Pathology groups) received an equivalent dosing volume (2 mL/kg) of 0.5% Klucel (one group with and one group without iron supplemented feed) and served as the controls. The main study and Pathology animals were treated once daily on Days 7 through 70 pp. Following this, Pathology animals were sacrificed while main study animals continued on study without receiving treatment (i.e., recovery phase) and underwent additional behavioral and fertility testing.

Doses: ICL670 was administered orally by gavage as suspensions in 0.5% (w/v) hydroxypropylcellulose (Klucel, aqueous solution) to four groups of pups (40 pups/sex/group for Main study groups and 12 pups/sex/group for Pathology groups) at doses of 10 (with iron supplemented feed), 20 (with iron supplemented feed) and 40 mg/kg/day (with and without iron supplemented feed). An additional 2 groups (40 pups/sex main study or 12 pups/sex Pathology groups) received an equivalent dosing volume (2 ml/kg) of 0.5% Klucel (one group with and one group without iron supplemented feed) and served as the controls. The main study and Pathology animals were treated once daily on Days 7 through 70 pp. Single-dose animals (N= 8/sex control or 16/sex/group) were used for toxicokinetics (TK) analysis after receiving one dose on Day 7 pp.

Basis of Dose Selection: Doses of 10, 20 and 40 mg/kg/day were selected were based on the results of an oral neonatal and juvenile development dose range-finding study in rats (Study 0370003) as described before. In this dose-ranging study, ICL670 was administered at 30 mg/kg/day (with iron supplemented feed), 60 mg/kg/day (with iron supplemented feed), 60 mg/kg/day (without iron supplemented feed), 90 mg/kg/day (with iron supplemented feed) and 90 mg/kg/day (without iron supplemented feed) from postpartum day 7 through day 34. Due to treatment-related mortality and moribundity, the groups at doses $\geq$ 60 mg/kg/day were terminated early (starting on Day 16 pp). Based on these results, a high dose of 40 mg/kg/day was selected for this study in order to have some evidence of toxicity in the absence of mortality; the dose of 10 mg/kg/day was expected to be a no adverse effect level (NOAEL) and the dose of 20 mg/kg/day was selected to examine the dose response.

Species/Strain: Rat/Wistar Hannover, ~ WI: ~ (BRL/Han) IGS BR

Number/Sex/Group or Time Point (Main Study): 40/sex/group

Route, Formulation, Volume: Oral, Suspension in 0.5% w/v Klucel, 2 ml/kg

Satellite Groups Used for Toxicokinetics: Single-dose animals (N = 8/sex control or 16/sex/group) were used for TK analysis after receiving one dose on Day 7 pp.

Age: Pregnant females were 12 weeks old. Pups were on *post partum* Day 7.

Weight: Neonatal body weights ranged from 10 to 20 grams for males and 12 to 20 grams for females at the initiation of treatment.

Sampling Times:

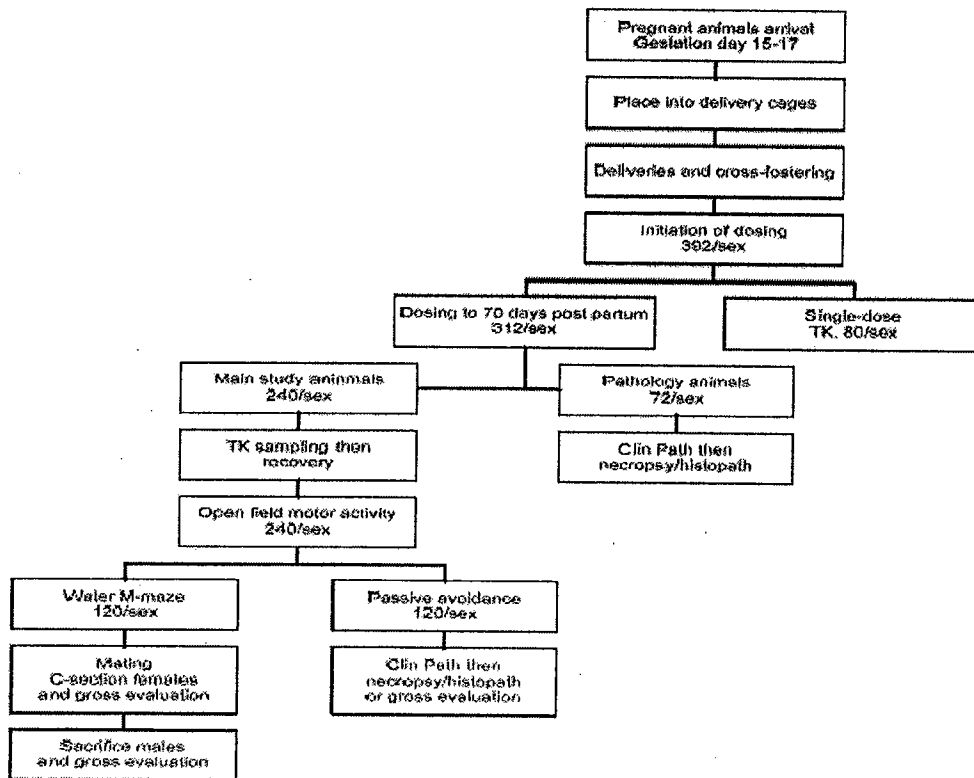
Single-dose animals: Blood samples were collected on post partum Day 7 from 4 pups/sex (2 pups/sex for control) at the following time points: 0.5, 2, 6 and 24 hours after the single dose. Maternal animals from the Single-dose groups were euthanized after the last blood collection and discarded without evaluation.

Main study animals: Blood samples were obtained at the end of the dosing phase (post partum day 75) at 0.5, 2, 6 and 24 hours after the final dose. Three blood samples per sex were collected at each time point.

Unique Study Design or Methodology: The following diagram shows the study design (from page 22 of the study report).

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Figure 3-1 Summary of the neonatal and juvenile development study design



The following table shows the details of the study design (from page 23 of the study report).

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Table 3-1 Study design, animal allocation and test article doses

Group	Number/group		Animal numbers ^{a,b}		Dose (mg/kg/day)	Concentration (mg/mL)
	male	Female	Male	female		
Single-dose animals						
1-Control ^f	8	8	2-16	1-15	0	0
2- Control	8	8	26-40	25-39	0	0
3 -Low ^c	16	16	50-80	49-79	10	5
4- Mid ^c	16	16	90-120	89-119	20	10
5-High ^c	16	16	130-160	129-159	40	20
6- High	16	16	170-200	169-199	40	20
Main Study animals						
1 - Control ^e	40	40	402-480	401-479	0	0
2- Control	40	40	490-568	489-567	0	0
3- Low ^f	40	40	578-656	577-655	10	5
4- Mid ^c	40	40	666-744	665-743	20	10
5-High ^d	40	40	754-832	753-831	40→30 ^d	20→15 ^d
6-High	40	40	842-920	841-919	40→30 ^{e,d}	20→15 ^{e,d}
Pathology animals						
1-Control ^f	12	12	210-232	209-231	0	0
2-Control	12	12	242-264	241-263	0	0
3- Low ^f	12	12	274-296	273-295	10	5
4- Mid ^c	12	12	306-328	305-327	20	10
5-High ^c	12	12	338-360	337-359	40→30 ^d	20→15 ^d
6-High	12	12	370-392	369-391	40→30 ^{e,d}	20→15 ^{e,d}

^aPSA computer assigns odd numbers to females and even numbers to males.

^bTo facilitate computer processing, separate studies were set up on the PSA computer system for processing main study animals by sex (0370030M and 0370030F), pathology study animals (0370030P) and the Single-dose males and females utilized for toxicokinetic measurements together (0370030T). Once dosing ended for main study animals, a new PSA study was used for processing males (0370030R) and females (0370030S). The day of bodyweight and food consumption collection was standardized for all animals following the last day of dosing. Histological pathology studies were set up on the PLACES system. Clinical pathology studies were set up on the [] Path/Tox system.

^cGroups 1, 3, 4 and 5 received iron supplemented feed. Groups 2 and 6 received standard diet.

^dDue to excessive mortality, the dose level and concentration were lowered beginning on 21-Oct-2003.

^eDue to excessive mortality, group 6 animals were terminated early on 22-Oct-2003. Animals in this group were not dosed on 22-Oct-2003.

Observation and Times:

Clinical Signs: Mortality and clinical signs were observed twice daily.

Body Weights: Main study animals: Body weights were recorded on post partum Days 0, 4, daily on Days 7 through 21, post partum Day 24, and then twice weekly on Days 28, 31, 35, 38, etc. until the end of dosing.

Food Consumption: Main study animals: Food consumption was recorded weekly after weaning on post partum Days 28, 35, 42, 49 until terminal sacrifice.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted during the first week after weaning on all surviving control (Groups 1 and 2) and high-dose (Group 5) main study animals and at the end of the dosing period on all main study animals.

Post-Weaning Evaluations: Surviving Main study animals were selected for one of two learning/memory tests (passive avoidance or water M- maze). The first, third, fifth, etc. animal in each group was assigned to the water M- maze. The remaining animals were assigned to passive avoidance testing. This resulted in approximately 20 animals/sex/group for each learning/memory test.

Hematology: Hematology assessments were conducted from blood samples collected from pathology animals at the end of the dosing period.

Clinical Chemistry: Clinical chemistry assessments were conducted from blood samples collected from pathology animals at the end of the dosing period.

Urinalysis: Urinalysis was conducted from blood samples collected from pathology animals at the end of the dosing period.

Gross Pathology: Necropsies were performed on all surviving pathology animals and the first ten surviving males and females in each dose group of Main study animals not assigned to fertility assessment.

Organ Weights: The following (from page 31 of the study report) organs were weighed from first ten surviving males and first ten surviving females of main study animals not assigned to fertility assessment in Groups 1 to 5, for all pathology animals at necropsy and for all unscheduled deaths/sacrifices.

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Tissue list: for Collection, Weighing (W) and/or Processing (P)

W	P	adrenal	W	P	ovary
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
W	P	brain	W	P	pituitary
	P	cecum	W	P	prostate
	P	cervix		P	rectum
	P	colon		P	salivary gland
	P	duodenum		P	sciatic nerve
	P	epididymis		P	seminal vesicle
	P	esophagus		P	skeletal muscle
	P	eye		P	skin
	P	femur/tibia		P	spinal cord
	P	harderian gland	W	P	spleen
W	P	heart		P	sternum
	P	ileum		P	stomach
	P	jejunum	W	P	testis
W	P	kidney	W	P	thymus
	P	lacrimal gland	W	P	thyroid
W	P	liver		P	tongue
	P	lung		P	trachea
	P	lymph node – bronchial		P	urinary bladder
	P	lymph node – mandibular	W	P	uterus
	P	lymph node – mesenteric		P	vagina
	P	mammary gland		P	macroscopic lesions
	P	nasal passage			animal identification

Histopathology: The tissues specified in the above list were examined histopathologically from all Pathology animals in the control and high dose groups (Groups 1, 2, 5) and from target organs (kidneys and eyes) and gross lesions in the low and mid-dose groups. For all Pathology animal unscheduled deaths/sacrifices as well as Pathology Group 6 early group termination sacrifices, histopathologic examinations was performed for all tissues listed. There was no tissue processing or microscopic examination performed for Main study early death/sacrifices. For Main study animals sent to Pathology at the end of the study, only target organs (kidneys and eyes) and gross lesions were processed for histopathologic examinations.

Adequate Battery: yes (X), no ()
Peer review: yes (), no (X)

Reproductive Parameters at terminal Cesarean-Section: For pregnant animals sacrificed on GD13, the number of corpora lutea and uterine implant site were examined.

Toxicokinetics:

Single-dose animals: Blood samples were collected on post partum Day 7 from 4 pups/sex (2 pups/sex for control) at the following time points: 0.5, 2, 6 and 24 hours after the single dose. Maternal animals from the Single-dose groups were euthanized after the last blood collection and discarded without evaluation.

Main study animals: Blood samples were obtained at the end of the dosing phase (post partum day 75) at 0.5, 2, 6 and 24 hours after the final dose.

Results:

Mortality: Treatment-related mortality was observed at 40 mg/kg/day in both the iron supplemented group (Group 5) and the non-iron supplemented group (Group 6) beginning on post partum Day 17. The high dose was reduced from 40 to 30 mg/kg/day on post partum Days 18 to 20 for these groups (Main study as well as Pathology animals). Despite the dose reduction, mortality in the Main study non iron supplemented high dose group (Group 6) exceeded 50% for males and 43% for females and this group was terminated early on 22- Oct- 2004. The following table (from page 35 of the study report) shows the mortality data prior to Group 6 termination.

Table 4-1 Summary of main study animal mortality prior to group 6 termination

Main study	Dose level	Male mortality	Female mortality
Group 5	40→30 mg/kg/day, with Fe supplemented feed	5/40	7/40
Group 6	40→30 mg/kg/day, without Fe supplementation	20/40	17/40

Following the reduction in the dose and after weaning, Group 5 had one additional female death (animal no. 791) which occurred during the recovery phase. Two other non-treatment-related deaths occurred in the Main study animals. One control male was found dead after being observed for several days with a crooked spine and another male was sacrificed due to a mechanical injury. Treatment-related mortality was also observed in the Pathology animals, with 1 of 12 males died at 40 mg/kg/day with iron supplementation and 5 of 12 male and 5 of 12 females were found dead at 40 mg/kg/day without iron supplementation prior to termination of Group 6. The cause of death was identified microscopically as damage to kidney tubules. In addition, there was one additional death in one Pathology group control female. There was no test article-related mortality at doses $\leq$ 20 mg/kg/day.

Clinical Signs: Treatment-related distended abdomens were noted in Main study and Pathology animals of both sexes at 40→30 mg/kg/day independent of the iron status. In addition, the following clinical signs were observed at 40→30 mg/kg/day without iron supplementation: recumbency and piloerection noted in a few males; pale and cool to

touch noted in a few females; and hunched posture noted in one animal of each sex in the Pathology animals. Test article-related ocular opacities (cataracts) were observed in 9 males at 40→30 mg/kg/day with iron supplementation and were considered treatment-related lenticular changes which developed during the treatment period. In addition, an ocular opacity was also observed in one female at 10 mg/kg/day.

Body Weights: The mean initial (Day 7) and final (Day 70) body weights of the control (Group 1, + Fe) males were 16 and 309 g, respectively. The mean initial (Day 7) and final (Day 70) body weights of the control (Group 1, + Fe) females were 15 and 191 g, respectively. Treatment-related reductions in body weight were observed at 40→30 mg/kg/day with and without iron supplementation when compared to control in males (86-94% of control) and females (84-95% of control). The reductions began at about day 16 postpartum for both groups and continued until study termination of Group 6.

Food Consumption: The mean initial and final food consumption in control (Group 1) males were 19 and 25 g/animal/day, respectively. The mean initial and final food consumption in control females were 17 and 19 g/animal/day, respectively. A decrease (88.2% of control) in food consumption was noted for post partum days 28 to 35 in females at 40→30 mg/kg/day with iron supplementation (Group 5).

Ophthalmoscopy: Ophthalmoscopic examinations revealed treatment-related findings mainly in the lens. These effects were observed in 1 of 39 females at 20 mg/kg/day and in all males (35 of 35) and 29 of 33 females at 40→30 mg/kg/day (Group 5). Group 6 animals (animals receiving 40→30 mg/kg/day without iron supplementation) died or were euthanized prior to ophthalmic examinations.

At post partum Weeks 3 and 4, ophthalmoscopic examinations revealed bilateral mature cataracts in 3 of 35 males while 1 of 33 female showed early bilateral cataract (cortical striations, vacuoles etc.) at 40→30 mg/kg/day with iron supplementation. At Week 9, there was an increase in the incidence and progression of these cataracts. Ophthalmoscopically, these changes were characterized as a circular opacity surrounding the equatorial cortex of the lens affecting 1 of 39 females at 20 mg/kg/day to hypermature cataracts whose cortex had liquefied allowing the lens nucleus to sink to the bottom of the lens (morgagnian cataract) affecting 35 of 35 males and 29 of 33 females at 40→30 mg/kg/day (Group 5).

During recovery period (Days 35 and 36, post partum Weeks 15 and 16), an increase in the number of circular opacities surrounding the equatorial cortex of the lens (zone of discontinuity) was seen in 4 of 32 females at 40→30 mg/kg/day (Group 5, +Fe). All other lenticular changes remained the same showing no detectable progression from the Week 9 examinations. There were no treatment-related lenticular effects noted in males at 20 mg/kg/day or at 10 mg/kg/day in either sex.

Post-Weaning Evaluations: No treatment-related effects were observed for the following tests conducted during the post weaning treatment period: acoustic startle, vaginal

opening, preputial separation, or pupillary reflex in females. There were no differences in motor activity, passive avoidance testing and water M- maze testing.

Fertility and C-Section: No significant effects were observed on mating or fertility index. However, there were increases in the pre- (7.5, 8.2, 3.3, 4.7 and 11.8% in Groups 1, 2, 3, 4, and 5, respectively) and post-implantation (6.8, 6.4, 4.8, 4.9 and 10.1% in Groups 1, 2, 3, 4, and 5, respectively) loss at 40→30 mg/kg/day with iron supplementation (Group 5). There were no reproductive effects observed at doses ≤ 20 mg/kg/day.

Hematology: There were no significant treatment-related changes.

Clinical Chemistry: There were no appreciable treatment-related findings.

Urinalysis: No treatment-related changes were observed.

Gross Pathology: Treatment-related macroscopic findings included discoloration (paleness) of kidneys at 40→30 mg/kg/day, which correlated with microscopic findings, in both sexes in Pathology as well as Main study animals. The incidence was markedly higher in animals fed a non iron supplemented diet. The incidence was as follows: Pathology animals: one male on iron-supplemented diet, 8 males and 7 females on non supplemented diet; Main Study animals: 2 females on iron supplemented diet, 3 males and 7 females on non supplemented diet. In addition, discoloration of adrenal glands was also observed at 40→30 mg/kg/day.

Organ Weights: There was a slight increase in mean absolute and relative (to body and brain) heart weights of males (absolute: 121% of control, control = 0.922 g; relative to body weight = 110.8% of control, control = 0.3236%; relative to brain weight = 118.8% of control, control = 44.432%) at 40→30 mg/kg/day in the Pathology animals group and females at the same dose in the Main study group. There were no other significant changes.

Histopathology: Treatment-related histopathology changes were mainly observed in the kidney (inflammatory and degenerative changes consisting of tubular vacuolation and/or necrosis at 40→30 mg/kg/day in males and females in the Pathology group. Additional changes at 40→30 mg/kg/day included tubular dilation and/or basophilia). Microscopic examinations of eyes did not reveal any treatment-related changes. The following table shows the histopathological lesions in the kidney from the Pathology and Main Group animals.

Tissue Findings	MALES (N = 12)						FEMALES (N = 12)					
	1	2	3	4	5	6	1	2	3	4	5	6
KIDNEY												
PATHOLOGY GROUP												
Vacuolation, cytoplasm, tubular					1	1						3
Necrosis, tubular						2						2
Mitosis, tubule						1						2
Inflammation, pelvis, neutrophilic							1					

Inflammation, interstitial, chronic						3						4
Infiltrate, leukocyte, interstitial					1						1	
Dilatation, tubular						4						6
Dilatation, pelvis	3	3	1		4	4		2			1	7
Degeneration, tubular						1						2
Basophilia, tubular						2	4				1	5
MAIN STUDY ANIMALS												
KIDNEY	MALE (N = 10)						FEMALE (N = 10)					
Inflammation				1	5						2	
Dilatation, pelvis		2	2	1	1							
Chronic progressive nephropathy					4						2	
Basophilia, tubular											1	

- *: Group 1: Control 1 (+Fe)
- Group 2: Control 2 (standard feed)
- Group 3: 10 mg/kg/day (+Fe)
- Group 4: 20 mg/kg/day (+Fe)
- Group 5: 40→30 mg/kg/day (+Fe)
- Group 6: 40→30 mg/kg/day (standard feed)

Toxicokinetics: Based on the dose normalized AUC_{0-24h} values, the exposure to ICL670 appeared to be over proportional between 10 and 40 mg/kg/day in both sexes on post partum Day 7 and in males on pp Day 75. There was no apparent sex-related difference in exposure on pp Day 7 at 10 and 20 mg/kg/day; however, females appeared to be more exposed to ICL670 at 40 mg/kg/day. At post partum Day 75, the exposure was 2- to 3-fold higher in females than in males at 10 and 20 mg/kg/day; however, there was no apparent sex difference in exposure at 40→30 mg/kg/day. Exposure decreased at the end of dosing amounting 4-13%, 12-22% and 17-11% of the exposure on post partum Day 7 at the low, mid and high dose levels, respectively. The mean toxicokinetic parameters are shown in the following table (from page 43 of the study report).

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Table 4-6 Mean toxicokinetic parameters of total ICL670 in rat plasma

Parameter (units)	Post partum Day 7							
	Group 3 – Iron + 10 mg/kg/day		Group 4 – Iron + 20 mg/kg/day		Group 5 – Iron + 40 mg/kg/day*		Group 6 – Iron – 40 mg/kg/day**	
	M	F	M	F	M	F	M	F
t_{max} (h)	2	2	2	6	6	2	2	6
C_{max} (µg/mL)	2.07	2.38	4.25	4.87	10.9	14.7	10.7	13.2
$C_{max}/Dose$ (µg/mL)/(mg/kg/day)	0.207	0.238	0.213	0.244	0.273	0.368	0.268	0.330
$AUC_{(0-24h)}$ (µg.h/mL)	26.9	25.9	58.7	66.8	145	197	152	196
$AUC_{(0-24h)}/Dose$ (µg.h/mL)/(mg/kg/day)	2.69	2.59	2.94	3.34	3.63	4.93	3.79	4.90
Parameter (units)	Post partum Day 75							
	Group 3 – Iron + 10 mg/kg/day		Group 4 – Iron + 20 mg/kg/day		Group 5 – Iron + 40→30 mg/kg/day*			
	M	F	M	F	M	F		
t_{max} (h)	2	0.5	0.5	0.5	2	0.5		
C_{max} (µg/mL)	0.360	0.826	2.90	6.93	3.09	3.64		
$C_{max}/Dose$ (µg/mL)/(mg/kg/day)	0.0360	0.0826	0.145	0.347	0.103	0.121		
$AUC_{(0-24h)}$ (µg.h/mL)	1.09	3.43	7.11	14.9	18.0	15.9		
$AUC_{(0-24h)}/Dose$ (µg.h/mL)/(mg/kg/day)	0.109	0.343	0.356	0.746	0.600	0.530		

* Groups 5 received 40 mg/kg/day from post partum day 7 to postpartum days 18-20 and thereafter switched to 30 mg/kg/day until day 70

** Group 6 terminated between postpartum days 19 and 21

Serum and Tissue Fe Analysis: At the end of dosing, male rats at ≥ 20 mg/kg/day showed decreased serum UIBC (universal iron binding capacity) and increased serum Fe concentrations. At the end of the treatment period, liver iron content was decreased (Male: 87.3, 96.9 and 81.7% of control, control = 197 mg/kg; Female: 80.4, 73.8 and 72.2% of control, control = 669 mg/kg at 10, 20 and 40→30 mg/kg/day, respectively) in all ICL670-treated groups when compared to iron supplemented control values. The kidney iron content was consistently altered compared to control. While heart iron content in males was not affected by the test article, however, females showed a somewhat dose-related decrease (91%, 82% and 89% of control, control = 112 mg/kg) in heart iron content at the end of dosing. At the end of the recovery period, the iron content of the liver was still reduced in both sexes. Kidney and heart iron values at the end of the study were not appreciably different from that of the control.

Summary: In an oral toxicity study in juvenile rats, animals were treated with ICL670 at 0, 10, 20 and 40→30 mg/kg/day from post partum day 7 through 70. Mortality was observed at 40 mg/kg/day irrespective of iron supplementation. The target organ of toxicity appeared to be the eye (cataract) and kidney (inflammatory and degenerative changes consisting of tubular vacuolation and/or necrosis and tubular dilation and/or basophilia at 40→30 mg/kg/day in males and females). The NOAEL appeared to be 20 mg/kg/day in males and 10 mg/kg/day in females. It is to be mentioned here that the NOAEL identified in the adult rat was 100 mg/kg/day. Therefore, the juvenile rats appeared to be approximately 5-10-fold more sensitive to the toxicity of ICL670 when compared to adults.

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Drug Batch: CGP 72670, Lot No. 800196.

Methods: Marmosets (2/sex/group) received ICL 670 orally, by gavage, at doses of 100, 200 or 400 mg/kg/day for 14 days. The dose volume was 5 ml/kg. The control group received 0.45% hydroxypropylmethylcellulose (HPMC) and polyethylene glycol 400 (PEG 400). The doses were selected based on the earlier studies (detailed of those studies were not mentioned). Animals were monitored twice daily for clinical signs and moribundity. Each animal was weighed at weekly intervals during the pretest and the treatment period. Food consumption was recorded on a daily basis during the pretest and treatment period. Hearing test and ophthalmoscopy was performed prior to treatment and on Day 15. Hematology and clinical chemistry and urinalysis were performed on pretest and Day 15. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroid. Histopathological examinations were performed on adrenals, aorta, brain, cecum, colon, duodenum, epididymides, eyes and optic nerves, femur, gall bladder, heart, ileum, jejunum, kidneys, knee-joint, liver, lungs, lymph nodes (axillary and mesenteric), mammary gland, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerves, seminal vesicles, skeletal muscles (thigh), skin, spinal cord (cervical, thoracic and lumbar segments), spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix and vagina.

Results:

1. **Clinical Signs:** Slight to moderate dose-related incidence of vomiting was observed in males at 200 and 400 mg/kg/day and in females at 400 mg/kg/day. Discolored urine was observed in males receiving 400 mg/kg/day. A possible treatment-related incidence of diarrhea/soft feces was recorded in all treated groups.
2. **Mortality:** None.
3. **Body Weight and Food Consumption:** The mean initial and final body weights of control males were 396.5 and 393 g, respectively. The mean initial and final body weights of control females were 402 and 411 g, respectively. Slight decrease in body weight was observed in males (89% of control). The numerical data for food consumption was not provided; however, the sponsor stated that the food consumption was unaffected by treatment with ICL 670.
4. **Hearing:** No treatment-related finding was observed.
5. **Ophthalmoscopy:** No treatment-related change was observed.
6. **Hematology:** One male at 400 mg/kg showed lower RBC (53% of control; control mean RBC = $8.22 \times 10^{12}/L$), hemoglobin (56% of control; control = 17.1 g/dL), hematocrit (58% of control; control = 0.565 %). An increase in WBC count (168% of control; control = $11.3 \times 10^9/L$) was seen in one male at 400 mg/kg/day. The percent neutrophil values were increased (183% of control; control = 38%) in both males at 400 mg/kg/day.
7. **Clinical Chemistry:** No treatment-related changes were observed.

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- 8. **Urinalysis:** No treatment-related changes were observed.
- 9. **Organ Weights:** No dose-related trends in either absolute or relative organ weights were observed.
- 10. **Gross Pathology:** No considerable macroscopic findings related to treatment were observed.
- 11. **Histopathology:** Treatment-related changes were observed in the kidneys and gall bladder. Two males and one female at 200 mg/kg/day and one male and two females at 400 mg/kg/day showed cortical proximal tubular epithelial vacuolation in the kidney. One male marmoset in each group at 200 and 400 mg/kg/day showed minimal to moderate vacuolation of the gall bladder epithelium.

12. Toxicokinetics in a 2-Week Oral Toxicity Study in Marmosets:

Total plasma concentrations of ICL 670 and its iron complex in marmosets were determined after oral administration of 100, 200 and 400 mg/kg (2/sex/dose) as single and repeated doses during a 2-week oral toxicity study. The following table provides mean plasma concentration values as mentioned above:

Dose (mg/kg)	Mean Max. Conc. in Males (µg/ml)		Mean Max. Conc. in Females (µg/ml)	
	Single Dose	Week 2	Single Dose	Week 2
100	62.7	87.8	27.2	48.3
200	126	116	143	131
400	128	166	153	126

In females, concentrations of ICL 670 increased overproportionately between 100 and 200 mg/kg dose levels after single dose; however, this increase was found to be less than proportional after single dosing in males. The accumulation in plasma was found to be low and there was no trend towards a sex-related difference in plasma concentrations of ICL 670.

In a 2-week oral gavage toxicity study in marmoset monkeys, animals received 0, 100, 200 and 400 mg/kg/day of ICL 670 by oral gavage for 14 days. The NOEL was established as 100 mg/kg/day. The target organs of toxicity appeared to be the kidney (cortical proximal tubular epithelial vacuolation at 200 and 400 mg/kg/day in both sexes) and gall bladder (minimal to moderate vacuolation of the gall bladder epithelium at 200 and 400 mg/kg/day in males).

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2-Week Oral Toxicity Study in Iron-Overloaded Marmosets (Study No. 971053)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland

Date Started: September 29, 1997

Date Completed: October 27/28, 1997

GLP Compliance: Statements of compliance with GLP regulations and Quality Assurance Unit were included. However, the statement states that the study did not fully comply with GLP principles, as there was no independent monitoring of the various phases of the study or the report by the Quality Assurance Unit.

Animals: Marmoset monkeys (*Callithrix jacchus*) were used throughout the study. Animals were 2.7-13.5 years old at the start of dosing and body weight ranges from 0.28-0.55 kg.

Drug Batch: ICL 670, Lot No. 800196.

Methods: The purpose of this study was to examine the hypothesis that the increased incidences of kidney proximal tubular cell vacuolization seen in a previous 2-Week study (study no. NVR015/971974) in marmosets would not occur in iron-overloaded monkeys. Animals (10/sex/group) were given three intraperitoneal injections (at fortnightly intervals) of iron (III) hydroxide poly-isomaltose (first and second injections: 200 mg/kg; third injection: 100 mg/kg). Group 1 (10/sex/group) received control vehicle (0.45% w/v hydroxypropylmethyl cellulose (HPMC) and 40% w/v of polyethylene glycol (PEG) 400. Group 2 animals received ICL 670 at 400 mg/kg/day for 2 weeks by oral gavage at a dose volume of 5 ml/kg. Animals were monitored for clinical signs twice daily. Body weights were recorded on Days 1, 3, 5, 8, 10, 12 and 14 of the treatment period. Food consumption was determined on a qualitative basis daily. Hematology and clinical chemistry were performed at pretest and before necropsy. Urinalysis was done on Day 11/12. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroids, uterus, etc. Histopathological examinations were performed on adrenal, aorta, bone/bone marrow, brain, cecum, colon, duodenum, epididymis, esophagus, eyes with optic nerve, gall bladder, heart, ileum, jejunum, kidneys, knee joint, lacrimal glands, liver, lung, lymph node (axillary, mandibular and mesenteric), ovaries, pancreas, pituitary gland, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin/mammary area, spinal cord, spleen, stomach, testes, thigh muscle, thymus, thyroid/parathyroid gland, tongue, trachea, urinary bladder, uterus and vagina.

Results:

1. **Clinical Signs:** No treatment-related clinical signs were observed at any time during the treatment.
2. **Mortality:** None.

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3. **Body Weight and Food Consumption:** The mean initial and final body weight of control males were 356.7 and 367.3 g, respectively. The mean initial and final body weight of control females were 352.8 and 370.2 g, respectively. There were no significant treatment-related alterations of body weight in male (95% of control). The body weight of treated females were significantly increased (108-114% of control) at various time points compared to control. Food consumption data were not provided in this report.
4. **Hematology:** No treatment-related changes were observed.
5. **Clinical Chemistry:** Bilirubin concentration was found to be elevated in males (40%) and females (53%) compared to control (male: 9.38 $\mu\text{mol/L}$; female: 8.54 $\mu\text{mol/L}$). There were no other treatment-related changes in the clinical chemistry parameters.
6. **Urinalysis:** The urine of 3/5 animals was found positive for leukocytes compared to control.
7. **Organ Weights:** No treatment-related changes were observed.
8. **Gross Pathology:** No treatment-related changes were observed.
9. **Histopathology:** There were no histological alterations related to treatment. However, deposition of iron due to iron pre-loading was observed in several organs including bone marrow, kidneys, large and small intestine, liver, lungs, lymph nodes, spleen, cells of the reticuloendothelial systems, etc. Histopathological changes in the kidney (chronic interstitial nephritis characterized by lymphohistiocytic and plasma cell infiltration, tubular degeneration, atrophy associated with regeneration and glomerulonephritis), liver (diffuse iron deposition, inflammation, etc.), spleen (diffuse iron deposition) were observed in both control and treated rats and considered to be due to iron-overloading or spontaneous lesion common to marmosets.
10. **Toxicokinetics:** The iron-preloaded marmosets (4 male and 4 female) received 400 mg/kg of ICL 670 once daily by oral gavage for two consecutive weeks. Concentrations of ICL 670 were determined on Day 1 and Day 12 by HPLC technique. Maximum plasma concentrations of 79.1 $\mu\text{g/ml}$ and 55.7 $\mu\text{g/ml}$ reached at 2 hr post dose on Day 1 in males and females, respectively. On Day 12, C_{max} of 72.7 $\mu\text{g/ml}$ and 79.8 $\mu\text{g/ml}$ was observed at 2 hr after treatment in males and females, respectively. No sex-related difference in mean plasma concentrations was observed.

In a 2-week toxicity study in iron pre-loaded marmosets, animals were treated with 400 mg/kg of ICL 670 by oral gavage. The NOEL appeared to be 400 mg/kg. There were no treatment-related clinical findings or overt toxicity findings. Histopathological alterations in the kidney (chronic interstitial nephritis characterized by lymphohistiocytic and plasma cell infiltration, tubular degeneration, atrophy associated with regeneration and glomerulonephritis), liver (diffuse iron deposition, inflammation, etc.), spleen (diffuse iron deposition) were observed in both control and treated rats. No target organs of toxicity could be identified in the absence of any treatment-related histopathological changes. ICL 670 at 400 mg/kg appeared to be well tolerated in the iron pre-loaded marmosets under the conditions of the study.

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4-Week Oral Toxicity Study in Marmosets Followed by a 4-Week Recovery Period (Report NVR/023/974194)**Testing Laboratory:** E J**Date Started:** August 26, 1997**Date Completed:** September 25, 1997**GLP Compliance:** Statements of compliance with GLP regulations and the Quality Assurance Unit were included.**Animals:** Marmoset monkeys were used throughout the study. Animals were approximately 1.25 – 3.6 years old and weighed between 297 – 548 g on the day of the first treatment.**Drug Batch:** ICL 670. Batch No. 800196.

Methods: Marmosets (4/sex/group) received ICL 670 by oral gavage at 25, 65 and 135 mg/kg/day for 4 weeks at a dose volume of 5 ml/kg body weight. Control animals received the vehicle (0.45% w/v HPMC/40% w/v PEG 400). The dose selection was based on the results of a 2-week oral toxicity study in marmosets (report no. NVR0015/971974). In this study, animals (2/sex/group) were treated with ICL 670 orally at doses of 100, 200 and 400 mg/kg for 2 weeks. Treatment-related changes were observed in the kidneys (cortical proximal tubular epithelial vacuolation) in both sexes at 200 and 400 mg/kg dose levels. The no observed adverse effect level NOAEL was established to be 100 mg/kg and the target organ of toxicity appeared to be kidney. Based on the results of this study, oral doses of 25, 65, and 130 mg/kg were suggested for one-month toxicity study in marmosets.

In the 4-week oral toxicity study in marmosets, recovery phase animals (2/sex/group) were treated for 28 days and were then allowed to recover for 28 days following the completion of the treatment. Animals were monitored for clinical signs daily. Body weights were recorded weekly during the treatment. Food consumption were recorded daily during treatment and recovery phase. Ophthalmoscopic examinations and hearing tests were also performed before and during week 4 of the treatment. Electrocardiograms (ECGs) were recorded during pre-treatment and week 4 of the treatment. Blood samples were taken for hematology and clinical chemistry at pre-treatment and during week 4 of the treatment. Urinalysis was performed at pre-treatment and week 3 of the treatment. Absolute and relative organ weights were determined for the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thyroid, and uterus. Histopathological examinations were performed on the adrenals, aorta, brain, bronchi, cecum, cervix, colon, duodenum, epididymis, eyes and optic nerve, femur, gall bladder, heart, ileo-caecal junction, ileum, jejunum, kidneys, lacrimal glands, liver, lungs, lymph nodes (bronchial, mandibular and mesenteric), mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (submandibular and parotid), sciatic nerve, seminal vesicles, skeletal muscles (thigh), skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

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Results:

1. **Clinical Signs:** There were no treatment-related clinical signs.
2. **Mortality:** There was no treatment-related mortality.
3. **Body Weight and Food Consumption:** The mean initial and final body weights of control males were 380 and 370 g, respectively. The mean initial and final body weights of control females were 398 and 392 g, respectively. The body weights of animals at 130 mg/kg/day were found to be 95% and 93% of control in males and females, respectively at week 4. The numerical data for food consumption were not provided in this report; however, the sponsor stated that there were no treatment-related alterations of food consumption.
4. **Ophthalmoscopy:** Normal.
5. **Hearing Examination:** Normal.
6. **ECG Examination:** Normal.
7. **Hematology:** Normal.
8. **Serum Chemistry:** A dose-related increase in plasma iron levels was observed in males (141%, 225% and 445% of control (18.93 $\mu\text{mol/L}$) at 25, 65, and 130 mg/kg, respectively) and females (140%, 165%, and 340% of control (21.78 $\mu\text{mol/L}$) at 25, 65, and 130 mg/kg, respectively) received ICL 670. There were no other findings considered to be treatment-related.
9. **Urinalysis:** Normal.
10. **Organ Weights:** Normal.
11. **Gross Pathology:** Normal.
12. **Histopathology:** Treatment-related histopathological changes were seen in the kidneys (vacuolar degeneration of cortical tubules in one out of four males at 130 mg/kg and protein cast in cortical tubules was seen in 2/4 males at 130 mg/kg), liver (moderate vacuolation of the intrahepatic bile duct cells in 1/4 males at 130 mg/kg), gall bladder (acute inflammation, vacuolar hyperplasia of the epithelium and fibrosis of wall of gall bladder in 1/4 males at 130 mg/kg). Cerebral mineralization was observed in 2/4 and 1/4 females at 130 and 65 mg/kg, respectively. Adrenal cortical hemorrhage was observed in 1/2 females at 25 and 65 mg/kg dose levels. Following a 4-week recovery period, histopathological changes observed in the kidney (protein casts in renal cortical tubular cells) in one male at 130 mg/kg were still evident suggesting that tubular damage may persist for a prolonged time period. One female animal at 130 mg/kg showed marked adrenal cortical hemorrhage at the end of the 4-week recovery period.

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13. **Toxicokinetics:** Total concentration of ICL 670 and its iron complex was determined in plasma on Days 1 and 28 of the repeated exposure to marmosets (3/sex/dose) at 25, 65, and 130 mg/kg dose. Concentrations were determined by HPLC technique. The pharmacokinetic parameters of total ICL 670 are summarized in the following table (from vol. 20, page 8-5237 and 8-5238 from sponsor's submission):

Time	Dose (mg/kg)	Male Marmosets			Female Marmosets		
		Cmax	Tmax	AUC	Cmax	Tmax	AUC
Day 1	25	10.5γ6.9	0.5*	54.8γ20.8	6.73γ1.17	0.5*	32.8γ5.5
	65	34.6γ7.5	2*	194γ19	13.1γ0.5	0.5*	81.9γ7.3
	130	70.8γ21.9	2*	445γ57	27.1γ11.3	0.5*	202γ37
Day 28	25	8.26	0.5*	49.	5.55	0.5*	41.5
	65	19.7γ4.0	2*	209	28.2γ8.8	2*	300γ53
	130	47.6γ20.8	0.5*	522γ286	50.3	2*	657γ142

*: Median value.

Cmax (µg/mL); Tmax (hr); AUC (0-24h) (µg.h/mL).

In a 4-week oral toxicity study in marmoset monkeys, animals received ICL 670 at doses of 25, 65, and 130 mg/kg/day. Two animals/sex/group entered a 4-week recovery period following treatment. A no observed effect dose (NOEL) was not established in this study; however, no-observed-adverse-effect level (NOAEL) was established as 65 mg/kg and 130 mg/kg in males and females, respectively. The target organs of toxicity appeared to be kidney (vacuolar degeneration and protein casts in cortical tubules in males at 130 mg/kg), liver (vacuolation of intrahepatic bile duct cells in ¼ male at 130 mg/kg) and gall bladder (inflammation, vacuolar hyperplasia of epithelium and fibrosis of wall of gall bladder in ¼ male at 130 mg/kg), brain (cerebral mineralization females at 65 and 130 mg/kg) and adrenal glands (adrenal cortical hemorrhage in females at 25 and 65 mg/kg). Protein casts seen in a single male at 130 mg/kg was still evident at the end of the 4-week recovery period and a single female at 130 mg/kg dose level showed adrenal cortical hemorrhage at the end of the 4-week recovery period.

4-Week Oral (Gavage) Palatability and Toxicity Study in Marmosets (Report No. NVR030/983573)

Testing Laboratory: []

J

Date Started: June 30, 1998

Date Completed: July 1, 1998

GLP Compliance: A statement of compliance was included.

Animals: Marmoset monkeys were used throughout the study. Animals were 1.5-2.5 months old and body weight ranges from 320 and 478 g on the day of the first dose.

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Drug Batch: ICL 670, Batch No. 97902.

Methods: The purpose of this study was to assess the palatability of an iron-supplemented diet and its effect on the systemic toxicity of ICL 670. In this study, two groups (2 male marmosets/group) of marmoset monkeys received 200 mg/kg/day of ICL 670 by oral gavage daily for 4 weeks. One of the treated groups received iron-supplemented diet (iron concentration = 3700 mg/kg diet). Two control groups received vehicle (0.45% w/v HPMC/40% w/v PEG 400) only and one of the control groups received normal diet and the other group was fed with iron-supplemented diet. Animals were monitored for clinical signs of toxicity and morbidity/mortality daily. Body weight was recorded weekly. Food consumption was recorded daily. Hematology and clinical chemistry parameters were studied at pretest and during week 4 of the treatment. All animals were subjected to a detailed necropsy and the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, lungs, pituitary, prostate, salivary glands, spleen, testes, thymus, and thyroid with parathyroid. Histopathological examinations were done on adrenals, aorta, brain, cecum, colon, duodenum, epididymides, eyes and optic nerves, femur, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), salivary glands, esophagus, pancreas, pituitary, prostate, rectum, sciatic nerve, seminal vesicles, skeletal muscles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, and urinary bladder.

Results:

1. **Observed Effects:** Animals receiving ICL 670 and iron-supplemented diet had diarrhea/soft feces from day 8 or 9 of treatment. Black feces were observed in animals receiving iron-supplemented diet.
2. **Mortality:** One animal was killed *in extremis* on Day 12 (lethargic, hunched posture, piloerection and eyes were partially closed).
3. **Body Weight:** The mean initial and final weights of the control animals (normal diet) were 392.5 and 377.5 g, respectively. The mean initial and final weights of the control animals with iron-supplemented diet were 353.5 and 338 g, respectively. The body weight of animals at 200 mg/kg of ICL 670 with normal diet was found to be 101% of control at week 4. The body weight of animals at 200 mg/kg of ICL 670 with iron-supplemented diet was 99% of control at week 4.
4. **Food Consumption:** The initial and final food consumption in control animals (normal diet) was 36.9 and 42.2 g/animal/day. The initial and final food consumption in control animals (with iron-supplemented diet) was 59.9 and 44.9 g/animal/day. The food consumption in animals at 200 mg/kg/day of ICL 670 with normal diet was found to be 117% of control at week 4. The food consumption in animal at 200 mg/kg/day of ICL 670 and with iron-supplemented diet was 41% of control at week 4.
5. **Hematology:** No significant treatment-related changes were observed.

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6. **Clinical Chemistry:** High plasma iron concentrations were observed in all animals received ICL 670 at 200 mg/kg/day (greatest in animals with normal diet and 200 mg/kg/day of ICL 670).
7. **Organ Weights:** Absolute liver weight was higher (158% of respective control) in animals at 200 mg/kg/day of ICL 670 with normal diet when compared to respective control (14.5 g). In case of animals at 200 mg/kg/day of ICL 670 and with iron-supplemented diet was 132% of respective control (control = 13.2 g).
8. **Gross Pathology:** There were no treatment-related changes were observed.
9. **Histopathology:** Histopathological findings were observed in the gall bladder (acute inflammation, increased mitotic index of epithelial cells and vacuolation of the epithelial cells), liver (neutrophil infiltration, inflammatory infiltrate in the portal areas and hemosiderin in the Kupffer cells), colon (acute inflammation and ulcer), rectum (acute inflammation and ulcer), spleen (congestion) and kidneys (dilatation of the cortical and medullary tubules and collecting ducts) of the animals given ICL 670 with iron-supplemented diet. Animals receiving iron-supplemented diet tolerated treatment less well compared to the animals on normal diet.
10. **Toxicokinetics:** Two male marmosets per group (receiving an iron-supplemented diet) were administered with ICL 670 once daily by gavage at a dose of 200 mg/kg for 4 weeks. The toxicokinetic parameters of ICL 670 are summarized in the following table (vol. 14, page 8-3254 of sponsor's submission):

Animal No.	Normal Diet			Iron Supplemented Diet		
	Cmax	Tmax	AUC	Cmax	Tmax	AUC
5	96.1	2	1070			
6	48.4	2	622			
7				29.4	2	247

Cmax (µg/ml); Tmax (hr); AUC (0-24hr) (µg.h/ml).

Maximum plasma concentration was reached at 2 hours post dose. The Cmax and AUC values of the iron-supplemented group were lower than those in the group receiving a normal diet. However, no definite conclusion was drawn from this study because of the high variability in the normal diet group and only one animal in the iron-supplemented diet group.

In a 4-week oral palatability and toxicity study in marmosets, animals (normal and iron-supplemented diet) were administered with ICL 670 at 200 mg/kg/day orally. The NOEL was not established. The target organ of toxicity appears to be gall bladder (acute inflammation, increased mitotic index of epithelial cells and vacuolation of the epithelial cells), liver (neutrophil infiltration, inflammatory infiltrate in the portal areas and hemosiderin in the Kupffer cells), colon (acute inflammation and ulcer), rectum (acute inflammation and ulcer), spleen (congestion) and kidneys (dilatation of the cortical and medullary tubules and collecting ducts) in the animals given ICL 670 with iron-supplemented diet. Animals receiving iron-supplemented diet tolerated treatment less well compared to the animals on normal diet.

Study Title: 2-Week Intravenous Toxicity Study in the Marmoset Monkeys

Study No: 0320068

Volume #, and Page #: Vol. 2 of 2, page # 1

Conducting Laboratory and Location:

Date of Study Initiation: November 12, 2003

Date of Study Completion: December 17, 2003

GLP Compliance: This is a draft report and the GLP compliance statement was not signed.

QA Report: yes () no (X)

Drug, Lot #, and % Purity: ICL670, Batch No. 4469, and

Formulation/Vehicle: Solution/Polyoxyl 35 castor oil (Cremophor EL, 65% w/v) and ethanol.

Methods: The purpose of this study was to determine the toxicity of ICL670 in marmosets after prolonged i.v. infusion and to determine the MTD of ICL670 following i.v. administration in the marmoset. This study has two phases: MTD phase and repeat dose phase. ICL670 was supplied as a solution in Cremophor EL and ethanol, was diluted with 5% glucose solution. In the MTD phase, the drug solution was administered to marmosets (n = 1/sex/group) as a slow continuous i.v. infusion (11.67 mL/kg, 1 mL/min) via tail vein at 0 and 10 for 3 days. Vehicle control animals received the reference item (placebo for ICL670: a solution of Cremophor EL and ethanol that was diluted with 5% glucose solution). In the repeat dose phase, animals received the ICL670 or placebo formulations by slow intravenous injection (1 mL/min) via the tail or saphenous vein at varying dose volumes at 0, 2, 5, and 10 mg/kg/day for 14 days. The following examinations were performed: mortality, clinical signs, body weight, ophthalmoscopy, electrocardiography, clinical pathology, organ weights, gross and microscopic examinations and toxicokinetic measurements.

Dosing:

Species/Strain: *Callithrix jacchus* (Marmoset monkeys)

No./Sex/Group or Time Point (Main Study): 3/sex/group

Satellite Groups used for Toxicokinetics or Recovery: 2/sex/group

Age: 18-30 months

Weight: Males: 313-389 g; Females: 324-443 g

Doses in Administered Units:

MTD Phase: 0, 10 mg/kg/day for 3 days

Repeat Dose Phase: 0, 2, 5, and 10 mg/kg/day for 14 days

Route, Form, Volume, and Infusion Rate: Intravenous, solution, varying dose volumes as shown in the following table. The infusion rate is 1 mL/kg.

Study Design

Group No.	Treatment	Dose (mg/kg/day)	Dose Volume (ml/kg)	Animal No. (Males)	Animal No. (Females)
MTD Phase					
1	ICL670	10	11.67	1	3
2	Control	0	11.67	2	4
Repeat Dose Phase					
1	Control	0	11.67	5, 6, 7	23, 24, 25
2	ICL670	2	2.33	8, 9, 10	26, 27, 28
3	ICL670	5	5.83	11, 12, 13	29, 30, 31
4	ICL670	10	11.67	14, 15, 16	32, 33, 34
Toxicokinetics					
2	ICL670	2	2.33	17, 18	35, 36
3	ICL670	5	5.83	19, 20	37, 38
4	ICL670	10	11.67	21, 22	39, 40

Observations and times:

Clinical Signs: Animals were observed twice a day.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was not recorded.

Ophthalmoscopy: Both eyes were examined at predose and on Day 10.

Electrocardiography: Electrocardiogram traces were recorded from all sedated main study animals during predose, and approximately 1 hour after dosing on Days 2 and 10 for the Limb Lead II.

Hematology: Blood samples for hematology were collected from all main study animals at predose and on Day 9 of treatment.

Clinical Chemistry: Blood samples for hematology were collected from all main study animals at predose and on Day 9 of treatment.

Urinalysis: Urine samples were collected from all main study animals during predose and on Day 13 of treatment over a 4 hour period.

Gross Pathology: Gross pathology was conducted at necropsy from all MTD and repeat dose study animals.

Organs Weighed: The following organs were weighed from all animals: adrenals, brain, heart, kidneys, liver, lungs and bronchi, ovaries, pancreas, pituitary, prostate, spleen, testes and epididymides, thymus, thyroid and parathyroid, uterus and cervix.

Histopathology: The following organs and tissues were used for histopathology from all animals from the MTD and the main study group: abnormal tissues, adrenals, aorta, brain, cecum, colon, duodenum, esophagus, eyes with optic nerves, gall bladder, heart, ileum, injection site, jejunum, kidneys, liver, lungs and bronchi, mammary gland, marrow smear, lymph nodes (mesenteric, submandibular, submaxillary), microchip, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, sciatic nerve, seminal vesicles, skeletal muscles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus with cervix, and vagina.

Toxicokinetics: Blood samples were obtained from satellite animals on Days 1 and 14 at the following time points: 0.5, 1, 2, 6 and 24 h postdose. Samples were analyzed for ICL670 by LC-MS/MS method.

Results:

Mortality: There were no unscheduled deaths in this study.

Clinical Signs: On Day 2 and 3, slight body tremors were observed during and after treatment in both animals in the MTD phase. In the main study, slight tremors were also observed at 5 and 10 mg/kg/day.

Body Weight: The mean initial and final body weight of control males were 332 and 323 g, respectively. The mean initial and final body weight of control females were 348 and 345 g, respectively. There were no treatment-related changes in either sex.

Food Consumption: Food consumption was not recorded.

Hematology: Hematological examinations on Day 9 revealed no changes that could be attributed to ICL670.

Clinical Chemistry: There were no treatment-related changes in clinical chemistry performed on Day 9.

Urinalysis: Urine composition was unaffected by ICL670 treatment.

Ophthalmology: There were no treatment-related findings.

Electrocardiography: There were no treatment-related findings.

Organ Weights: There were no treatment-related changes on organ weights.

Gross Pathology: There were no treatment-related macroscopic findings.

Histopathology: Treatment-related histopathological changes were observed in the thyroid (focal lymphocytic infiltration), liver (focal necrosis, bile duct hyperplasia, focal hemorrhage) and kidney (inflammation). Although, there is no obvious dose response relationship, however, histopathological changes seen in the thyroid, liver and kidney could be attributed to the treatment, as these changes were not observed in any control animal. Treatment-related histopathological changes are shown in the following table.

Tissue Finding	Males (n = 3)				Females (n = 3)			
	0 mg/kg	2.0 mg/kg	5.0 mg/kg	10 mg/kg	0 mg/kg	2.0 mg/kg	5.0 mg/kg	10 mg/kg
Thyroid:								
Lymphocytic infiltration	0	0	2	2	0	0	0	1
Liver:								
Necrosis, focal	0	0	2	1	0	1	0	0
Bile duct hyperplasia, focal	0	0	1	1	0	1	0	0
Hemorrhage, focal	0	0	2	1	0	0	0	0
Kidney:								
Inflammation	0	1	1	2	2	1	1	0

Toxicokinetics: No toxicokinetic analyses were performed for the low dose group due to low sample volume availability. The exposure to total ICL670 increased slightly over dose proportionately between 5 and 10 mg/kg/day at Day 1 and Day 14. Exposure appears to be higher in males than females. The mean toxicokinetic parameters are shown in the following table (Table 2-2, Vol. 2, page 10 of sponsor's submission).

Table 2-2 Mean toxicokinetic parameters of total ICL670 in marmoset plasma

		Day 1			
		Intermediate dose group (5 mg/kg/day)		High dose group (10 mg/kg/day)	
Parameter	Units	Male	Female	Male	Female
T _{max}	h	0,5	0,5	0,5	0,5
C _{max}	µg/mL	6,40	4,98	17,1	11,0
C _{max} /Dose	(µg/mL)/(mg/kg/day)	1,28	0,995	1,71	1,10
AUC _(0,5-24h)	µg.h/mL	8,26	6,04	28,2	18,7
AUC _(0,5-24h) /Dose	(µg.h/mL)/(mg/kg/day)	1,65	1,21	2,82	1,87

		Day 14			
		Intermediate dose group (5 mg/kg/day)		High dose group (10 mg/kg/day)	
Parameter	Units	Male	Female	Male	Female
T _{max}	h	0,5	0,5	0,5	0,5
C _{max}	µg/mL	7,20	5,84	20,9	15,1
C _{max} /Dose	(µg/mL)/(mg/kg/day)	1,44	1,17	2,09	1,51

Summary: In this 2-week i.v. infusion study in marmosets, marmosets were treated with ICL670 at 2, 5, and 10 mg/kg/day for 2 weeks by slow i.v. infusion. The NOAEL could not be established in this study, as histopathological changes were observed at all dose levels. The MTD appeared to be 10 mg/kg/day in the absence of any body weight change or mortality. The target organs of toxicity appeared to be the thyroid (focal lymphocytic infiltration), liver (focal necrosis, bile duct hyperplasia, focal hemorrhage) and kidney (inflammation).

Study Title: 39-Week Oral Gavage Toxicity Study in Marmosets

Key Study Findings: In a 39-Week oral (gavage) toxicity study in marmoset monkeys, animals were treated with ICL670 at 20, 40 and 80 mg/kg/day. Mortality was observed at 80 mg/kg/day. Treatment-related findings in decedent animals on standard diet (two males and one female at 80 mg/kg/day) were observed in the gall bladder (acute inflammation and generalized degeneration and vacuolation of the hepatic bile duct) and kidneys (vacuolation and degeneration of the renal cortical tubules in all three animals and dilatation of the medullary tubules and Bowman's space in one male). The 40 mg/kg/day dose may be considered as the NOAEL.

Study No.: 982027

Volume #, and Page #: EDR 2002/Toxicology/98207, page 1

Conducting Laboratory and Location: ☐

1

Date of Study Initiation: December 1, 1998

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 98903, $\bar{C}$ 1

Methods: ICL670 was administered to groups of four male and four female marmosets at 20, 40 or 80 mg/kg/day (5 ml/kg), once daily, seven days a week, for 39 weeks. Control animals received the vehicle (0.225% hydroxypropyl methylcellulose/20% polyethylene glycol 400). A further two males and two females were assigned to each of the control and high dosage groups for the 4-week recovery phase of the study. An additional group receiving 80 mg/kg/day ICL670 was given an iron supplemented diet.

Doses: 20, 40 and 80 mg/kg/day

Species/strain: Marmoset monkeys (*Callithrix jacchus*)

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, and volume: Oral (gavage), suspension in 0.225% w/v hydroxypropyl methylcellulose (HPMC) and 20% v/v polyethylene glycol 400 (PEG 400), 5 ml/kg

Satellite groups used for recovery: Recovery: 2/sex/group

Age: 18-38 months old

Weight: 314 to 562 g

Sampling times: On day 1 and during Week 39 of treatment blood samples were obtained from each marmoset at 0.5, 2, 6 and 24 hours after dosing.

Study design: The study design is shown below (from page 19 of the study report).

3.3.3. Study design

Pretest period: At least 10 weeks.

Duration of dosing: 39 weeks.

Recovery period: 4 weeks.

Table 3.3-1. Study design, animal allocation and test article dosages

Group	Animal numbers		Recovery phase		Test article Dosage (mg/kg/day)
	Males	Females	Males	Females	
1	1279, 1283, 1293, 1301	1108, 1194, 1208, 1222	1275, 1299	1198, 1204	0
2	1285, 1289, 1303, 1327	1190, 1202, 1214, 1074			20
3	1281, 1307, 1313, 1315	1188, 1206, 1212, 1220			40
4	1277, 1287, 1291, 1297	1178, 1196, 1200, 1210	1197, 1311	1182, 1216	80
5 †	1201, 1209, 1213, 1225	1110, 1116, 1118, 1126			80

† Animals given iron-supplemented diet (3-fold normal iron concentration, added as Niferex® Elixir).

Observations and Times:

Mortality: Mortality was checked daily.

Clinical Signs: Clinical signs were observed daily.

Body Weights: Body weight was recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Both eyes of all marmosets were examined before commencement of treatment and during Weeks 13, 25 and 38 of treatment by means of a direct ophthalmoscope after instillation of 1.0% tropicamide. The structures examined included the following: adnexa, cornea and sclera, conjunctiva, lens and vitreous, anterior chamber and iris, and ocular fundus.

Electrocardiography: Electrocardiograph tracings were recorded from all marmosets before commencement of treatment for the three standard limb leads (1, 2 and 3) and the three augmented limb leads (aVR, aVL and aVF) at Weeks 12, 24 and 37. The following parameters were measured: heart rate wave intervals: P, PR, QRS, ST, QT amplitudes: P, Q, R, S, T. The QTc interval was calculated.

Hematology: Hematology examinations were conducted at pre-treatment and during Weeks 13, 26 and 38 of treatment (before administration) and during Week 4 of recovery.

Clinical Chemistry: Clinical chemistry was conducted at pre-treatment and during Weeks 13, 17, 26 and 38 of treatment (before administration) and during week 4 of recovery.

Urinalysis: Urinalysis was conducted at pre-treatment and during Weeks 12, 25 and 37 of treatment and Week 3 of recovery.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed: adrenals, lungs (including bronchi), testes, brain, ovaries, thymus, epididymides, pituitary, thyroids with parathyroids, heart, prostate, uterus with cervix, kidneys, salivary glands, liver and spleen.

Histopathology: the following organs/tissues (from page 26 of the study report) were examined from all animals:

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Tissue list

The following organs/tissues were taken from all animals:

adrenals	liver	skeletal muscle - thigh*
aorta	lungs	spinal cord
brain	lymph nodes: mandibular	spleen
bronchi	lymph nodes: mesenteric	sternal bone and marrow
caecum	mammary area - left	stomach
colon	oesophagus	testes
duodenum	optic nerves	thymus
epididymides	ovaries	thyroid with parathyroids
eyes	pancreas	tongue
femoral bone	pituitary	trachea
gall bladder	prostate	urinary bladder
heart	rectum	uterus with cervix
ileum	salivary glands	vagina
jejunum	sciatic nerves*	
kidneys	seminal vesicles	

* only one processed histologically.

Adequate Battery: yes (X), no ()

Peer Review: yes (X), no ()

Results:

Mortality: Three animals receiving the high dosage died or were sacrificed during the treatment period. Male No. 1291 (Group 4M, 80 mg/kg/day and given normal diet) died during Week 17. The clinical signs at Week 16 included thin build, hunched posture, piloerection and diarrhea. Its condition deteriorated rapidly during Week 17 and it became prostrate, with labored respiration and was cold to the touch. The animal died during the despatch procedure. Macroscopic findings at necropsy included a thin build and occasional soft, punctate masses in the gall bladder. Female No. 1110 (Group 5F, 80 mg/kg/day and given iron-supplemented diet) was killed in extremis in Week 18. Signs included thin build, hunched posture, underactive/lethargic behavior, pale gums and face and diarrhea. Male No. 1311 (Group 4M, 80 mg/kg/day and normal diet) was killed during Week 32 of treatment in moribund condition. Ante-mortem signs included underactivity, piloerection, hunched posture, very thin build, pale face and reduced body temperature (34°C). This animal had anemia. Macroscopic examination at necropsy revealed a markedly distended GI tract with abnormal contents (watery, no solid matter). The gall bladder wall was thickened and contained abnormal contents (yellow viscous fluid). The thyroids were enlarged.

Clinical Signs: Treatment-related clinical signs included thin build, piloerection, pale face and underactivity at 80 mg/kg/day. In addition, a high incidence of diarrhea was observed in males at 80 mg/kg/day (normal diet) and in females with iron-supplemented diet.

Body Weights: The mean initial and final body weight of control males were 422 and 442 g, respectively. The mean initial and final body weight of control females were 423 and 492 g, respectively. The final body weights of treated males were 99.1%, 98.8%, 98.2% and 87.3% of control at 20, 40 and 80 and 80 (+Fe, Group 5) mg/kg/day, respectively. The final body weight of treated females were 97.4%, 95.7%, 90.4% and 84.6% of control at 20, 40 and 80 and 80 (+Fe, Group 5) mg/kg/day, respectively.

Food Consumption: The mean initial and final food consumption in control males were 53.14 and 41.85 g/animal/day, respectively. The mean initial and final food consumption in control females were 55.14 and 48.42 g/animal/day, respectively. The final food consumption in treated males were 112.9%, 122.8%, 108.8% and 110.6% of control at 20, 40 and 80 and 80 (+Fe) mg/kg/day, respectively. The final food consumption in treated females were 105.8%, 107.3%, 116.6% and 94.9% of control at 20, 40 and 80 and 80 (+Fe) mg/kg/day, respectively.

Ophthalmoscopy: Ophthalmoscopic examinations at Weeks 13, 25 and 38 did not reveal any findings related to treatment.

Electrocardiography: Electrocardiograph investigations during Weeks 12, 24 and 37 did not reveal any changes attributable to treatment.

Hematology: Hematology examinations during Weeks 13, 26 and 38 of treatment revealed the following changes at 80 mg/kg/day (Group 4 and 5) when compared to control: slightly low packed cell volumes (male: 90% of control, control = 0.550 L/L; female: 94-95% of control, control = 0.516 L/L), hemoglobin concentrations (Male: 84-92% of control, control = 16.7 g/dl; Female: 90-91% of control, control = 16.0 g/dL) and red blood cell counts (male: 90-92% of control, control = $7.85 \times 10^{12}/L$; female: 94-96% of control, control = $7.22 \times 10^{12}/L$). During Week 4 of the recovery phase packed cell volume, hemoglobin concentration, red blood cell count and mean cell hemoglobin concentration were still low at 80 mg/kg/day.

Clinical Chemistry: Total protein concentrations were found to be low (90-92% of control, control = 71-77 g/L) during Week 13 at 80 mg/kg/day and iron-supplemented diet. Total protein and albumin concentrations were low during Weeks 17 and 26 at 80 mg/kg/day and given either normal or iron-supplemented diet and during Week 38 in males at 80 mg/kg/day and iron-supplemented diet and in females at 80 mg/kg/day and normal diet. In addition, high urea concentrations were also observed during Weeks 26 and 38 in females at 80 mg/kg/day and either normal or iron-supplemented diet.

Urinalysis: There were no significant treatment-related changes.

Gross Pathology: Macroscopic findings observed for animals sacrificed during the treatment period included a thin build and occasional soft, punctate masses in the gall bladder for male No. 1291 (80 mg/kg/day and normal diet) which died in Week 17. Female No. 1110 (80 mg/kg/day and iron-supplemented diet) which was killed in Week 18 had a thin build and pale kidneys. Male No. 1311 (80 mg/kg/day and normal diet) which was killed in Week 32 had a markedly distended GI tract with abnormal contents (watery, no solid matter); the gall bladder wall was thickened and contained abnormal contents (yellow viscous fluid) and the thyroids were enlarged. There were no other significant findings.

Organ Weights: There were significant no changes that were clearly attributable to treatment during the treatment or the recovery phase.

Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), no ()

Treatment-related histopathological changes in the dead animals (two males and one female at 80 mg/kg/day) included generalized degeneration of the gall bladder in two males; vacuolation of the hepatic bile duct cells for all three animals; acute inflammation of the gall bladder for male No. 1311; vacuolation and/or degeneration of the renal cortical tubules for all three animals and dilatation of medullary tubules and Bowman’s space for male No. 1291. There were no significant treatment-related histopathological lesions in animals sacrificed at the end of the treatment period or the recovery period. Histopathological observations in three (two males and one female at 80 mg/kg/day on standard diet) decedent animals are shown in the following table.

Tissue Finding	Males (n = 2)					Females (n = 1)				
	1	2	3	4	5	1	2	3	4	5
Gall Bladder										
Generalized degeneration	0	0	0	2	0	0	0	0	0	0
Acute inflammation	0	0	0	1	0	0	0	0	0	0
Kidneys										
Chronic interstitial nephritis	0	0	0	1	0	0	0	0	0	1
Basophilic cortical tubules	0	0	0	0	0	0	0	0	0	1
Extramedullary hemopoiesis	0	0	0	1	0	0	0	0	0	0
Dilated cortical tubules	0	0	0	1	0	0	0	0	0	0
Papillary mineralization	0	0	0	1	0	0	0	0	0	0
Vacuolation of cortical tubules	0	0	0	2	0	0	0	0	0	1

Dilatation of Bowmans space	0	0	0	1	0	0	0	0	0	0
Dilatation of medullary tubules	0	0	0	1	0	0	0	0	0	0

*:

- Group 1: 0 mg/kg/day
- Group 2: 20 mg/kg/day
- Group 3: 40 mg/kg/day
- Group 4: 80 mg/kg/day
- Group 5: 80 mg/kg/day (+Fe)

Histopathological findings in animals sacrificed at scheduled sacrifice are shown in the following table.

Tissue Finding	Males (n = 4)					Females (n = 4)				
	1	2	3	4	5	1	2	3	4	5
Group*										
Colon: Lymphoid hyperplasia	0	0	0	0	0	0	0	1	0	0
Gall Bladder: Increased mitotic index	0	2	1	0	1	0	1	0	0	1
Kidney										
Chronic interstitial nephritis	3	2	3	2	3	3	3	1	2	3
Cortical fibrosis	0	0	0	0	0	0	0	0	1	0
Cortical tubular degeneration/regeneration	0	0	0	0	0	0	0	0	1	0
Liver										
Chronic inflammatory cells within the portal area	0	0	0	0	0	0	0	0	0	1

*:

- Group 1: 0 mg/kg/day
- Group 2: 20 mg/kg/day
- Group 3: 40 mg/kg/day
- Group 4: 80 mg/kg/day
- Group 5: 80 mg/kg/day (+Fe)

Toxicokinetics: The systemic exposure to total ICL670 was approximately proportional to the administered dose. There was no apparent significant difference in systemic exposure between Day 1 and Week 39 indicating negligible accumulation upon multiple exposures. There was no apparent sex-difference in the systemic exposure to total

ICL670. The mean toxicokinetic parameters of total ICL670 in animals given the test article are summarized in the following table (from page 38 of the study report).

Table 4.5-1. Summary of toxicokinetics of total ICL670 in the marmosets

C_{max} ($\mu\text{g/mL}$): highest observed plasma concentration; $t_{1/2}$ (h): time to reach C_{max} ; $\text{AUC}(0-24\text{h})$ ($\mu\text{g}\cdot\text{h/mL}$): area under the plasma concentration-time curve calculated from $t = 0$ to 24 h by the trapezoidal rule.

Sex	Dose (mg/kg)	Mean parameter: Day 1			Mean parameter: Week 39		
		C_{max}	$t_{1/2}$ *	$\text{AUC}(0-24\text{h})$	C_{max}	$t_{1/2}$ *	$\text{AUC}(0-24\text{h})$
M	20	5.92	0.5	30.4	8.40	0.5	47.6
F		4.07	0.5	25.3	5.85	0.5	38.3
M	40	12.2	1.25	61.2	15.5	0.5	69.5
F		13.8	2	72.7	12.9	0.5	91.4
M	80	25.3	0.5	165	23.9	0.5	145
F		21.3	1.25	127	25.4	0.5	139
M	80**	34.1	0.5	145	30.3	0.5	139
F		25.7	1.25	124	30.1	0.5	137

* Median value.

** Animals given iron supplemented diet.

Iron Analysis: Treatment with ICL670 decreased liver iron content by an average of 79%, 87% and 95% for males receiving normal diet at 20, 40 or 80 mg/kg/day, respectively, and by 67% and 82%, respectively, for females receiving normal diet at 40 or 80 mg/kg/day. Kidney iron content was also reduced for all treated male groups and females receiving 40 or 80 mg/kg/day.

Summary: In a 39-Week oral (gavage) toxicity study in marmoset monkeys, animals were treated with ICL670 at 20, 40 and 80 mg/kg/day. Mortality was observed at 80 mg/kg/day. Histopathological findings in animals (two males and one female at 80 mg/kg/day on standard diet) sacrificed or died during the treatment period indicated that the target organs of toxicity appeared to be the gall bladder (acute inflammation and generalized degeneration), hepatic bile duct (vacuolation) and kidneys (vacuolation and degeneration of the renal cortical tubules and dilatation of the medullary tubules and Bowman's space). The 40 mg/kg/day dose may be considered as the NOAEL.

2.6.6.4 Genetic toxicology

Study Title: Ames assay with ICL670 (Study No. CGP72670)

Methods: This is an exploratory study and was not conducted under GLP conditions. In this study, ICL670 (Batch No. 800196) was tested at 5-5000 $\mu\text{g}/\text{plate}$ using two strains TA98 and TA100 in the presence and absence of S9.

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Results: ICL670 did not precipitate up to 5000 µg/plate concentration. ICL670 showed cytotoxicity at concentrations of ≥ 3000 µg/plate, in strain TA100, and at 5000 µg/plate in strain TA98. ICL670 was not mutagenic in this assay.

Study Title: Ames Assay (Study No. 956192)

Methods: This is another exploratory study and the sponsor did not provide any statement of GLP compliance. In this study, ICL670 (Batch No. Charge C) was tested at 92.6-2500 µg/plate using TA100, TA1535, WP2uvra, TA98, TA1537 and TA102 in the presence and absence of S9.

Results: A reduction in the growth of the background lawn was observed with strains TA 100, TA 1535, TA 1537, TA 102 and *E. coli* WP2 uvrA at the highest concentration (2500 µg/plate). No increase in the number of revertant colonies was observed with any strain at any concentration tested. ICL670 was not mutagenic in this assay.

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GENOTOXICITY**Mutagenicity Test Using Salmonella (Study No. 981683)****Testing Laboratory:** Novartis Pharma AG
Basel, Switzerland.**Date Started:** March 06, 1998**Date Completed:** April 28, 1998**GLP Compliance:** Statement of compliance with GLP regulations and the Quality Assurance Unit were included.**Drug Batch:** ICL 670. Batch No. 97902.

Methods: The genotoxic potential of ICL 670 was examined using *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, TA100 and TA102. The S-9 metabolic activation system was prepared from livers of male rats treated once with Aroclor 1254 by the intraperitoneal route at dose of 500 mg/kg. In this assay, agar was mixed with the test substance, the bacterial culture, and the S-9 metabolic activation system (or buffer) and are plated together on a layer of minimal agar in Petri dishes. ICL 670 was tested at concentrations of 8 to 5000 µg/plate in the presence and absence of metabolic activation. The solvent controls were carried out both in the presence and absence of an S9-mix. Positive controls in the absence of metabolic activation were sodium azide with TA1535 and TA100, 9-aminoacridine with TA97a, 2-nitrofluorene with TA98 and mitomycin C with TA102. The positive control in the presence of metabolic activation was 2-aminoanthracene with TA1535, TA98, TA100, TA97a and TA102. Plates were incubated for 3 days at 37°C and relevant colonies were counted using a [] image analyzer.

Results: ICL 670 did not precipitate on the tester plates up to the concentration of 5000 µg/plate. The validity of the test result was checked by comparing the solvent control data with range of the historical control data. A test compound was considered to be mutagenic if it produces, in at least one concentration and one strain, a response equal to twice (or more) the control incidence. The only exception was TA102, which has a spontaneous revertant number of more than 200, and an increase by a factor of 1.5 above the control level is considered as an indication of mutagenic effect. ICL 670 was negative in the Salmonella test. The positive controls produced expected response with respective strains.

ICL 670 possessed no mutagenic potential in the Salmonella mutagenicity test under the conditions of the experiment described above.

Study Title: Micronucleus Test In Vitro with V79 Chinese Hamster Cells**Study No.:** 971709**Volume #, and Page #:** EDR/NDA21-882/Pharmtox/tox/971709**Conducting Laboratory and Location:** Novartis Pharma AG, Preclinical Safety, Toxicology/Pathology, Muttensz, Switzerland**Date of Study Initiation:** October 13, 1997

Date of Completion: January 16, 1998

GLP Compliance: A statement of compliance was included.

QA Reports: yes () no (X)

Drug, Lot #, and % Purity: ICL670, Batch No. 1. Purity data was not provided.

Methods:

Cell line: V79 Chinese Hamster cells

Doses used in definitive study: The following table (from page 4 of the study report) shows the concentration ranges tested.

2.2.4. Concentration ranges tested

Toxicity assay		Micronucleus assay	
(-S9)	78.1 to 5000.0 µg/ml	(-S9)	20 hr 40.0 to 150.0 µg/ml
(+S9)	78.1 to 5000.0 µg/ml	(+S9)	3 hr 40.0 to 1250.0 µg/ml
		(+S9)	3 hr 40.0 to 1250.0 µg/ml
		(-S9)	3 hr 2500.0 to 5000.0 µg/ml
		(+S9)	3 hr 400.0 to 2500.0 µg/ml

Basis of dose selection: Basis of dose selection was not provided.

Negative controls: MEM media

Positive controls: Ethylmethane sulfonate (5 mM) for -S9 and Cyclophosphamide (20 µM) for +S9

Incubation and sampling times: 3 hr, 20 hr (-S9), 3 hr (+S9) and sampling time: 48 hr

Results:

Study validity: The sponsor did not mention the criteria for a valid study. However, the study appeared to be valid as positive controls produced expected results. For each substance at least three concentrations (2 cultures per concentration) and 4000 cells per concentration were analyzed. A result was considered positive if micronucleus frequencies were $\geq 1\%$ and at least 0.6% above the concurrent solvent control value.

Study outcome: Positive. In the presence of S9 and in the absence of S9 after 20h treatment cell growth inhibition was observed at higher concentrations. After 3h treatment in the absence of S9, ICL670 was not found to be cytotoxic. In the presence of S9, ICL670 induced increased numbers of cells containing micronuclei. However, these effects were not reproduced in the two following experiments. Overall, ICL670 appeared

to be positive in this study, as per the criteria for a positive result (at least 0.6% above the concurrent solvent control value). The results are shown in the following table (from page 6 of the study report).

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Table 4.3.-1. Experiments 1 to 3

Experiment 1			
Treatment (20 hr, -S9)	% cells with MN	Treatment (3 hr, +S9)	% cells with MN
MEM	0.48	S9	0.53
77.5 µg/ml	1.00	396.9 µg/ml	1.44
96.5 µg/ml	1.00	704.3 µg/ml	1.17
120.3 µg/ml	0.65	1250.0 µg/ml	1.28
150.0 µg/ml	too toxic		
EMS, 5 mM	11.92	CP, 20 µM	5.33
Experiment 2			
Treatment (3 hr, +S9)	% cells with MN		
S9	0.28		
396.9 µg/ml	0.20		
704.3 µg/ml	0.35		
1250.0 µg/ml	0.28		
CP 20 µM	5.66		
Experiment 3			
Treatment (3 hr, -S9)	% cells with MN	Treatment (3 hr, +S9)	% cells with MN
MEM	0.20	S9	0.38
3988.5 µg/ml	0.50	1357.2 µg/ml	0.80
4454.5 µg/ml	0.28	1842.0 µg/ml	0.28
5000.0 µg/ml	0.40	2500.0 µg/ml	0.57
EMS, 15 mM	11.28	CP, 20 µM	8.45
Historical negative control data			
Treatment conditions	% MN cells ± SD	number of experiments	
-S9, 3 hr	0.54 ± 0.17	50	
-S9, 20 hr	0.60 ± 0.16	67	
+S9, 3 hr	0.61 ± 0.19	113	

Study Title: Micronucleus Test *In Vitro* with V79 Chinese Hamster Cells

Study No.: MIC 127

Volume #, and Page #: EDR/NDA21-882/Pharmtox/tox/MIC 127

Conducting Laboratory and Location: Novartis Pharma AG, Preclinical Safety, Toxicology/Pathology, Muttenz, Switzerland

Date of Study Initiation: Not provided

Date of Completion: September 18, 1998

GLP Compliance: A statement of compliance was included.

QA Reports: yes () no (X)

Drug, Lot #, and % Purity: ICL670, Batch No. 900196. Purity data was not provided.

Methods: In this study, V79 Chinese hamster cells were exposed to different concentration of the test or control articles in the presence or absence of S9. The cells were sampled at 48 hours after treatment. For each concentration, at least three concentrations (2 cultures per concentration) and 4000 cells per concentrations were analyzed.

Cell line: V79 Chinese Hamster cells

Doses used in definitive study: The following table (from page 4 of the study report) shows the concentration ranges tested.

Concentrations ranges tested:

Toxicity assay:

(-S9), 19.5 to 1248.0 µg/mL

(+S9), 19.5 to 1248.0 µg/mL

Micronucleus assay:

(-S9), 3h, 50.0 to 300.0 µg/mL

(+S9), 3h, 150.0 to 600.0 µg/mL

(-S9), 3h, 70.0 to 250.0 µg/mL

(+S9), 3h, 20.0 to 150.0 µg/mL

Basis of dose selection: Basis of dose selection was not provided.

Negative controls: MEM (Modified Eagle's Medium)/DMSO (Dimethylsulfoxide)

Positive controls: Ethylmethane sulfonate (15 mM) for -S9 and Cyclophosphamide (10 µM) for +S9

Incubation and sampling times: 3 hr, (-S9), 3 hr (+S9) and sampling time: 48 hr

Results:

Study Validity: The sponsor did not mention the criteria for a valid study. However, the assay appeared to be valid as positive controls produced expected results. For each substance at least three concentrations (2 cultures per concentration) and 4000 cells per

concentration were analyzed. A result was considered positive if micronucleus frequencies were $\geq 1\%$ and at least 0.6% above the concurrent solvent control value.

Study Outcome: Positive. ICL670 was precipitated in the medium at 1248 $\mu\text{g/ml}$ concentration. In the presence of S9, cell growth inhibition was observed at lower concentrations. In the absence of S9, cell growth inhibition was observed at higher concentration. ICL670 increased (0.73, 0.68, 0.63, 0.88 % for control, 9.0, 122.5 and 165.1 $\mu\text{g/ml}$, respectively in Experiment 1; 0.38, 0.95, 0.50 and 1.13% for control, 132.3, 163.6 and 202.2 $\mu\text{g/ml}$, respectively) number of cells containing micronuclei both in the absence and presence of S9. The results with S9 were not provided. The results are shown in the following table (from page 6 of the study report). ICL670 was found to be clastogenic in this assay. The results are shown in the following table (from page 8 of the study report).

Table 3-1 Experiment 1

Treatment (3h, -S9)	% Cells with MN	Treatment (3h, +S9)	% Cells with MN
MEM / DMSO	0.50	S9 / DMSO	0.73
9.0 $\mu\text{g/ml}$	0.78	28.0 $\mu\text{g/ml}$	0.66
122.5 $\mu\text{g/ml}$	1.03	39.1 $\mu\text{g/ml}$	0.63
165.1 $\mu\text{g/ml}$	1.10	54.8 $\mu\text{g/ml}$	0.88
222.6 $\mu\text{g/ml}$	too toxic	76.6 $\mu\text{g/ml}$	too toxic
EMS, 15 mM	6.33	CP, 10 μM	4.45

Table 3-2. Experiment 2

Treatment (3h, -S9)	% Cells with MN	Treatment (3h, +S9)	% Cells with MN
MEM / DMSO	0.70	S9 / DMSO	0.38
132.3 $\mu\text{g/ml}$	1.35	39.1 $\mu\text{g/ml}$	0.95*
163.6 $\mu\text{g/ml}$	1.28	54.8 $\mu\text{g/ml}$	0.50
202.2 $\mu\text{g/ml}$	1.43	76.6 $\mu\text{g/ml}$	1.13
250.0 $\mu\text{g/ml}$	too toxic	107.2 $\mu\text{g/ml}$	too toxic
EMS, 15 mM	12.10	CP, 10 μM	5.10

* 2000 cells counted

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In Vitro Chromosomal Aberration Test in Cultured Human Peripheral Blood Lymphocytes (Study No. 981803)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: March 10, 1998

Date Completed: May 26, 1998

GLP Compliance: Statement of compliance with GLP regulations and the Quality Assurance Unit were included.

Drug Batch: ICL 670, Batch No. 97902.

Methods: The genotoxic potential of ICL 670 was examined in a chromosomal aberration assay using phytohemagglutinin-stimulated human lymphocytes. The drug was tested in the presence and absence of S-9 metabolic activation system. The S-9 metabolic activation system was prepared from livers of male rats treated once with Aroclor 1254 by the intraperitoneal injection at dose of 500 mg/kg. The following table shows the summary of various treatment conditions:

Experiment	Type of Treatment	Treatment + Recovery Period (h)	S-9	Duration of Treatment (h)	Harvest Time (hours after start of treatment)
CA1	Continuous	20+0	-	20	20
CA1	Pulse	3+17	-	3	20
CA2	Continuous	44+0	-	44	44
CA2	Pulse	3+41	+	3	44
CA3	Pulse	3+17	-	3	20

The part of the experiment CA2 with S-9 had to be repeated in experiment CA4 since no metaphases were analyzable. In experiment CA5 the treatment time with S-9 (3+17) was repeated since no concentration group had a mitotic index close to 50%. ICL 670 was tested at 4.7×10^{-11} , 2.3×10^{-8} , 5.8×10^{-6} , 7.3×10^{-4} , 0.0456, 1.43, 23.0, 185.6, 742.6, 1485.2 $\mu\text{g/ml}$ in the absence of metabolic activation in experiment CA1 and CA3. In experiment CA1 with S-9 the following concentrations were used: 4.3×10^{-11} , 2.1×10^{-8} , 5.3×10^{-6} , 6.7×10^{-4} , 0.0415, 1.3, 21.0, 168.9, 657.7, 1351.4 $\mu\text{g/ml}$. The concentrations tested in experiment CA2 without S-9 were: 1.45, 2.20, 2.90, 4.39, 5.77, 8.75, 11.5, 17.4, 23.0 and 34.8 $\mu\text{g/ml}$. The concentrations of ICL 670 in experiment CA4 with S-9 are as follows: 2.6, 4.0, 5.3, 8.0, 10.5, 15.9, 20.9, 31.6, 41.6 and 63.1 $\mu\text{g/ml}$. The concentrations in experiment CA5 with S-9 were: 0.35, 1.4, 4.8, 14.1, 35.7, 77.2, 143, 227, 309.2, 360.4 $\mu\text{g/ml}$. Positive controls were ethyl methanesulfonate (EMS) in the absence of metabolic activation and cyclophosphamide (CP) in the presence of metabolic activation. Lymphocytes were obtained from healthy, non-smoking volunteers and cultured for approximately 46 hours at 37°C. Cells were treated as outlined in the above table. Cultures receiving continuous treatments retained treatment medium until harvest. Pulse treatments were for 3 hours only. Cells were then centrifuged (200xg, 10 minutes), washed twice with sterile

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saline and then resuspended in fresh medium containing fetal calf serum and gentamycin. The cells were arrested in metaphase by adding colcemide (0.2 µg/ml) approximately 2.5 hours prior to harvesting. The lymphocytes were then placed in hypotonic medium for 20 minutes and then fixed with ice-cold methanol:acetic acid (3:1, v/v). Metaphases were spread by air-drying. The slides were stained with Giemsa (2%) solution and mounted in Depex. The mitotic index was determined for each culture by examining 1000 lymphocytes. The cultures were evaluated for structural and numerical chromosome aberrations by examining 100 well-spread metaphases. The chi-square test was used to analyze the data.

Results: ICL 670 produced a concentration-dependent decrease in the mitotic index. Treatment with ICL 670 in the presence or absence of metabolic activation did not increase the frequency of cells with chromosomal aberrations at any concentration tested. The positive controls produced increased number of cells carrying chromosomal aberrations.

ICL 670 did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes under the test conditions.

Rat Bone Marrow Micronucleus Test by the Oral Route (Study No. 981866)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: April 22, 1998

Date Completed: June 26, 1998

GLP Compliance: Statement of compliance with GLP regulations and the Quality Assurance Unit were included but not signed by the appropriate authority.

Drug Batch: ICL 670, Batch No. 97902.

Animals: Wistar Hanover rats were used in this study. The animals were 8 weeks old at the beginning of the experiment and the males weighed 121-244 g and the females weighed 146 - 200 g.

Methods: The mutagenic potential of ICL 670 was evaluated by testing its potential to induce micronuclei in polychromatic erythrocytes (PE) in the bone marrow of Wistar rats. ICL 670 was administered to Wistar rats (5/sex/group) twice orally with an interval of 24 hours between two administrations at doses of 50, 158 and 500 mg/kg (dose volume 5 ml/kg). Positive control animals (5/sex) were treated orally with 20 mg/kg cyclophosphamide (CP) at a dose volume of 5 ml/kg. Negative control animals (5/sex) were treated with Klucel HF 0.5% (dose volume of 5 ml/kg). The dose selection was based on the results of a preliminary dose-range finding experiment where rats (4/sex) were treated twice with ICL 670 with an interval of 24 hours between two administrations with a single high dose (800 mg/kg). The animals were then monitored for signs of toxicity. Three males and two females died at this dose and showed strong signs of toxicity (labored breathing and pronounced sedation). At 500 mg/kg, the animals showed less severe similar clinical signs and no premature death was observed. On the basis of

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these results, doses of 50, 158, and 500 mg/kg were chosen for the micronucleus test. The animals were sacrificed 48 hours after the first treatment and bone marrow was collected from the distal end of the femur in a centrifuge tube. The polychromatic erythrocytes were collected by passing the bone marrow cell suspension through the cellulose column. The eluate containing the anucleated cells i.e. polychromatic erythrocytes (PE) was directly loaded onto 35% Percoll solutions, centrifuged, resuspended in fetal calf serum and flattened by [] using a [] air-dried at room temperature for 24 hours before staining. The slides were stained with Giemsa stains for automatic micronucleus analysis using image analyzer. The data were analyzed by PROTOX software package. A test compound was classified as mutagenic in this assay if it induced a micronucleus frequency statistically significantly above the control level.

Results: A dose-dependent decrease in the percentage of polychromatic erythrocytes was observed indicating strong bone marrow toxicity. The percentage of polychromatic erythrocytes were 57.3 γ 8.4%, 43.4 γ 18.9% and 13.8 γ 17.4% at 50, 158 and 500 mg/kg, respectively where the vehicle control was found to be 58.7 γ 9.7%. The mean values of micronucleated polychromatic erythrocytes (MPE) were 0.14 γ 0.05%, 0.22 γ 0.11% and 0.27 γ 0.11% at 50, 158 and 500 mg/kg compared to a negative control value of 0.14 γ 0.06%. The MPE value at 500 mg/kg was found to be significantly different from control. Two males and four females died at this dose level. The mean percentage of MPE in the positive control group was found to be 4.27 γ 1.15%.

In the *in vivo* rat bone marrow micronucleus test by oral route, ICL 670 was found to be positive at 500 mg/kg dose level. However, at this dose level 6 animals died and bone marrow toxicity was also observed at this dose level. This positive result may be attributed to the pharmacological action of the test compound (i.e. iron chelation) rather than a direct genotoxic mechanism.

Oral Bone Marrow Micronucleus Test in Rats (Preliminary Follow-Up Study, Study No. 981876)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: Not provided.

Date Completed: Not provided.

GLP Compliance: Not provided. The present study was conducted non-GLP with a non-standard protocol.

Drug Batch: ICL 670, Batch No. 97902.

Animals: Wistar Hanover male rats were used in this study. The animals were 9-10 weeks old at the beginning of the experiment and the males weighed 218-299 g.

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Methods: In the previous study (study no. 981866) discussed above, ICL 670 was found to be positive in rat bone marrow micronucleus test at 500 mg/kg dose level. This result indicated that the test compound has the potential to cause chromosomal damage or to affect the spindle apparatus at 500 mg/kg dose level. As ICL 670 is an iron-chelator, it was hypothesized that this positive response at 500 mg/kg dose may be due to the pharmacological action (iron chelation) of the test compound rather than a direct genotoxic effect. The present study was conducted non-GLP with a non-standard protocol to address this hypothesis. This study consisted of two parts: A) animals were treated twice with 500 mg/kg of ICL 670 and bone marrow sampled 1, 2, 3, 6 and 10 days after the second treatment to investigate the effect of bone marrow recovery on the test result and to select the most sensitive sampling time for additional study; B) animals were treated with 500 mg/kg of the iron complex of ICL 670, CGP 82813 and bone marrow was sampled at 1 day after the second treatment. Removal of nucleated cells from bone marrow, preparation of cell suspension, cyto-centrifugation (to flatten cells) and staining procedures are same as described above for the study number 981866.

Results: In the micronucleus test, there was a time-dependent recovery of polychromatic erythrocytes. At this dose level, 7 of 21 animals died prematurely. The percentages of polychromatic erythrocytes (PE) were $52.3 \pm 13.9\%$ in the control group, and $16.7 \pm 12.9\%$ (significant), $28.9 \pm 7.1\%$, $50.7 \pm 7.9\%$, $34.6 \pm 6.6\%$ and $64.9 \pm 2.7\%$ at 1, 2, 3, 6, and 10 days after the treatment, respectively. CGP82813 produced a PE value of $20.2 \pm 11.9\%$ (significant). The mean values of MPE were $0.13 \pm 0.03\%$ in the negative control ($5.37 \pm 1.35\%$ for positive control) and $0.35 \pm 0.11\%$ (significant), $0.36 \pm 0.07\%$ (significant), $0.44 \pm 0.27\%$ (significant), $0.76 \pm 0.32\%$ and $0.15 \pm 0.03\%$ at 1, 2, 3, 6, and 10 days time points, respectively. ICL 670 was found to be positive at 500 mg/kg dose. This result might indicate that the test compound, ICL 670, has the potential to cause chromosomal damage or to affect the spindle apparatus in rat bone marrow cells *in vivo* at lethal, strongly bone marrow toxic dose.

Oral Bone Marrow Micronucleus Test in Iron-Overloaded Rats (Follow-Up Study, Study No. 981878)

Testing Laboratory: Novartis Pharma AG
Basel, Switzerland.

Date Started: December 16, 1998

Date Completed: April 14, 1999

GLP Compliance: Statement of compliance with GLP regulations and the Quality Assurance Unit were included but not signed by the appropriate authority.

Drug Batch: ICL 670, Batch No. 98903.

Animals: Wistar Hanover male rats were used in this study. The animals were 9 weeks old at the beginning of the experiment and the males weighed 187-307 g.

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Methods: In two previous studies (study nos. 981866 and 981876), ICL 670 was shown to be positive in bone marrow micronucleus test at bone marrow toxic, lethal dose of 500 mg/kg indicating that the test compound has the potential to cause chromosome damage in rat bone marrow cells. The present study was conducted to examine the possibility that this weakly positive response of ICL 670 at 500 mg/kg dose level could be due to its pharmacological action (iron depletion) rather than a direct genotoxic mechanism. In the present study, the male rats were iron-overloaded by intraperitoneal injection of iron (III)-hydroxide (40 mg/kg twice per week) for 6 weeks. Following a two week interim phase, the animals were treated orally (two administrations with a 24-hour interval) with ICL 670 at 300 and 500 mg/kg doses (dose volume was 5 ml/kg). Cyclophosphamide (CP) was administered at 10 mg/kg dose level (dose volume 5 ml/kg) as a positive control. Bone marrow was sampled for micronucleus according to the procedure described above at 48 and 168 hours after the first treatment.

Results: At 500 mg/kg dose, 9/12 rats (non-iron-overloaded) died prematurely in the 48 hour sampling time group. In the 168 hour sampling time group, 6/8 animals (non-iron-overloaded) died. At 300 mg/kg dose, 1/7 (non-iron-overloaded) died prematurely in the 48 hour sampling time group. There was no death in the 168 hour sampling time group at 300 mg/kg. There was no death in the iron-overloaded animals at any dose level. ICL 670 treatment caused pronounced myelotoxic effects at both dose levels in iron-overloaded and non-iron-overloaded rats in the 48 hour sampling group. However, at 168 hour sampling group, bone marrow depression was only observed at 500 mg/kg dose in non-iron-overloaded rats. In the micronucleus assay, at 48 h sampling time, there was no significant increase in the frequencies of MPE over vehicle control at any doses irrespective of iron-overloading. This is in contrast to the previous study where ICL 670 was found to be positive at the 48 hour standard sampling time at 500 mg/kg dose in the non-iron-overloaded rats. However, the increase in MPE frequency in the first study was only by a factor of less than 2. At prolonged sampling time of 168 hour, a statistically significant increase in MPE frequency over control value (0.14 γ 0.04%) was observed at 500 mg/kg (0.58 γ 0.04%) in non-iron-overloaded rats. In iron-overloaded rats, no significant effect was seen at any dose level. The following table shows the summary of micronucleus test at 48 and 168 hour sampling time:

Treatment	Dose (mg/kg)	No. of Animals	Iron over-loading	Frequency (%)		
				MPE	MNE	PE
48 Hour sampling time						
Control	Vehicle	5	Yes	0.17 $\pm$ 0.02	0.08 $\pm$ 0.09	55.5 $\pm$ 9.9
ICL 670	300	5	Yes	0.20 $\pm$ 0.09	0.23 $\pm$ 0.13	8.8 $\pm$ 10.4
ICL 670	500	5	Yes	0.12 $\pm$ 0.04	0.10 $\pm$ 0.05	10.8 $\pm$ 6.0
CP	10	5	Yes	2.00 $\pm$ 0.36	0.42 $\pm$ 0.17	33.1 $\pm$ 6.9
Control	Vehicle	5	No	0.16 $\pm$ 0.05	0.02 $\pm$ 0.03	50.6 $\pm$ 12.9
ICL 670	300	5	No	0.20 $\pm$ 0.09	0.05 $\pm$ 0.04	10.2 $\pm$ 5.7
ICL 670	500	3	No	0.23 $\pm$ 0.06	0.05 $\pm$ 0.03	16.3 $\pm$ 15.9
CP	10	5	No	2.07 $\pm$ 0.34	0.37 $\pm$ 0.11	25.1 $\pm$ 3.2

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168 Hour sampling time						
Control	Vehicle	5	Yes	0.16±0.04	0.17±0.11	51.0±6.4
ICL 670	300	5	Yes	0.20±0.08	0.16±0.05	54.6±7.9
ICL 670	500	5	Yes	0.31±0.15	0.26±0.13	39.3±20.4
CP	10	5	Yes	0.40±0.10	0.15±0.09	59.2±5.4
Control	Vehicle	5	No	0.14±0.04	0.08±0.05	50.5±7.2
ICL 670	300	5	No	0.19±0.12	0.12±0.05	46.8±5.4
ICL 670	500	2	No	0.58±0.04	0.16±0.06	21.4±16.8
CP	10	5	No	0.15±0.04	0.05±0.02	45.9±10.0

MPE: Micronucleated polychromatic erythrocytes
 MNE: Micronucleated normochromatic erythrocytes
 PE: Polychromatic erythrocytes
 CP: Cyclophosphamide

ICL 670 was found to be negative at 500 mg/kg dose in iron-overloaded rats. At 300 mg/kg, ICL 670 was shown to be negative in rat bone marrow micronucleus test irrespective of iron over-loading of rats.

Induction of Micronuclei in Rat Liver (Report No. 1463/57-D5140)

Testing Laboratory: []

]

Date Started: July 9, 1998

Date Completed: December 29, 1998

GLP Compliance: Statement of compliance with GLP regulations and the Quality Assurance Unit were included.

Drug Batch: ICL 670, Batch No. 97902.

Animals: Wistar male rats were used in this study. The animals were 9 weeks old at the beginning of the experiment and weighed 219-259 g.

Methods: This study was conducted to examine the clastogenicity of ICL 670 *in vivo* by induction of micronuclei in the rat hepatocytes. The bone marrow micronucleus test was shown to be insensitive to a range of rat liver carcinogens and as bone marrow is a peripheral tissue, toxic effects are frequently observed at doses well below those, which result in significant concentrations in the bone marrow. However, the hepatocytes are normally non-dividing but they can be induced to proliferate by partial hepatectomy. Therefore, in the main study, rats (8/dose) were dosed twice (one at 0 hours before partial hepatectomy at 24 hours and another dose at 24 hours after partial hepatectomy). ICL 670 was tested in a rat hepatocyte micronucleus test at 125 and 250 mg/kg (oral gavage, 10ml/kg). The dose levels were selected based on the

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results of a preliminary study in which rats were administered twice (at 0 and 48 hours) with 300, 700 and 1000 mg/kg of ICL 670 by oral gavage. The animals were observed for 2 days following the second administration. This was followed by a surgical range-finding study (to confirm that the dose levels selected on the basis of toxicity could be tolerated in animals which had undergone partial hepatectomy) where each animal received test article twice (ICL 670) at 200, 350 or 500 mg/kg doses one day prior to and one day following partial hepatectomy. The animals were observed for 2 days following the second administration. Based on the results of these preliminary studies, 125 and 250 mg/kg doses were selected for the main study. The vehicle control was 0.5% (w/v) Klucel and dimethylnitrosamine (DMN) was used at 10 mg/kg dose as a positive control.

Rats were sacrificed at 72 hours or 96 hours after the first chemical treatment and liver was dissected out after perfusion. The hepatocytes were carefully teased out using a combing method. Hepatocytes were washed and suspended and placed on slides, hydrolyzed in 5M hydrochloric acid and stained and examined at 1000 x magnification and a minimum of 2000 cells were analyzed per animal.

Results: Clinical signs were observed at 72 and 96 hour after first dose of 125 mg/kg which included piloerection, hunched posture, red eye secretion and red staining on head, nose and mouth. At 250 mg/kg, similar clinical signs were observed at 72 hours after second treatment and one rat was found dead following the second administration. At 96 hours after the second treatment, animals (at 250 mg/kg) showed clinical signs which included lethargy, hunched posture, piloerection, eye closure, swollen abdomen, prostration, reduction in body temperature, loose feces, tremors, unsteady gait, red staining to nose and mouth, abnormal breathing, and lack of righting reflex. One animal was sacrificed *in extremis* prior to the second dose. One animal was killed *in extremis* and one animal found dead following the second administration.

ICL 670 did not cause significant increase in micronucleus frequency at any dose level at either sampling time compared to the vehicle control. However, ICL 670 caused a dose-related decrease in the group mean mitotic index. The following table summarizes the results of the induction of micronuclei in rat liver hepatocytes:

72 Hour sampling time						
Treatment (mg/kgx2)	Number of animals	Number of HEP analyzed	Micronucleated HEP	Micronucleated hepatocyte/1000 scored	Statistical significance	Group mean MI (%)
Vehicle	5	10000	20	2.0		0.65
125	5	10000	8	0.80	p≤0.05	0.50
250	5	10000	21	2.10		0.25
96 Hour sampling time						
Vehicle	5	10000	19	1.90		0.14
125	5	10000	24	2.4	NS	0.08
250	5	10000	31	3.10	NS	0.04
DMN	5	10000	129	21.5	p≤0.001	0.03

ICL 670 did not induce micronuclei in the hepatocytes of rats treated at 125 and 250 mg/kg dose levels.

Addendum to 981866: In the conclusion of the study 981866, it was stated that the positive results in *in vivo* micronucleus test may be attributed to the pharmacological action of the test compound (i.e., iron chelation) rather than a direct genotoxic mechanism. This is not substantiated that iron chelation has produced positive results.

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2.6.6.5 Carcinogenicity

Study Title: 2-Year Oral (Gavage) Carcinogenicity Study with ICL670 in Wistar Hannover Rats

Key Study Findings: In this study, no test article-related mortality or moribundity was evident upon histopathological assessment. At study termination, 72-84% survival was observed in the males and 58-68% survival was observed in the females. There were no statistical differences in survival between the control groups and ICL670-treated dose groups. There were no test article-related effects noted during palpable mass examination. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates. Based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks did not appear to be carcinogenic at doses up to 100→60 mg/kg/day.

Study Number: 017022

Volume #, and Page #: Page 1 of Report 017022

Conducting Laboratory and Location: Novartis Pharmaceuticals Inc., East Hanover, NJ

Date of Study Initiation: May 21, 2001

Date of Study Completion: September 27, 2004

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, ⌈ ⌋

CAC Concurrence: Yes. Exec CAC dated June 5, 2001 (Appendix-1)

Study Type: 2-Year bioassay

Species/Strain: Rats/Wistar Hannover

Number/Sex/Group; Age at Start of Study: 50/sex/group; Age: 8 weeks

Body Weight: Males: 204.5 to 316.8 g; Females: 148.3 to 209.1 g

Animal Housing: Animals were housed in pairs in stainless steel cage.

Formulation/Vehicle: Suspension in 0.5% (w/v) Klucel (type HF), NF

Drug Stability/Homogeneity: ICL670 suspensions in 0.5% Klucel, 0.75 mg/mL to 100 mg/mL, were found to be stable for at least ⌈ ⌋ stored at 6°C and for at least ⌈ ⌋ stirring at room temperature. The 0.5% Klucel was found to be suitable for use for at least ⌈ ⌋ stirring at room temperature and at least ⌈ ⌋ stored at 6°C. The uniformity of the dosing formulations was confirmed. Samples prepared for Weeks 1, 5, 9, 24, 39, 54, 69, 84, and 104 were tested and the concentrations were ⌈ ⌋ of target concentrations. Nine control samples were tested, and no ICL670 was detected.

Methods: The purpose of this study was to determine the potential carcinogenicity profile of ICL670, when administered orally to rats fed an iron supplemented diet for at least 104 weeks. ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/ kg/ day (feed supplemented with iron). Beginning pretest Day 8 (May 14, 2001), Group 1 controls received standard certified rodent diet and animals in Groups 2-5 received certified rodent diet supplemented with iron (approximately 1680 ppm ± 10%), *ad libitum*. ICL670 doses were decreased on Day 29 of the study based upon the recommendations of CDER ExecCAC (ExecCAC meeting minutes dated June 5, 2001, Appendix-1). The dosing volume for all dose groups was 5 mL/kg.

Doses: 0 (standard feed, Group 1), 0 (feed supplemented with iron, Group 2), 25→15 (feed supplemented with iron, Group 3), 50→30 (feed supplemented with iron, Group 4) and 100→60 mg/ kg/ day (feed supplemented with iron, Group 5)

The following table presents the study design (from page 19 of the study report)

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3.3.3 Study design

Pretest period: Approximately 2 weeks
 Duration of dosing: At least 104 weeks

Table 3-1 Study design, animal allocation and test article doses

Group	Number/sex	Animal Numbers		Dose (mg/kg/day)	Concentration (mg/mL)
		males	females		
1 Control (standard feed)	50	1001-50	1501-50	0	0
2 Control (iron supplemented feed)	50	2001-50	2501-50	0	0
3 Low (iron supplemented feed)	50	3001-50	3501-50	25→15*	5→3
4 Mid (iron supplemented feed)	50	4001-50	4501-50	50→30*	10→6
5 High (iron supplemented feed)	50	5001-50	5501-50	100→60*	20→12

*Dose decrease effective day 29.

Animals were fasted daily for approximately 4 hours, whenever possible, prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing, whenever possible. All changes to this intended schedule were acknowledged/approved and documented in the raw data file. Feeding/fasting times were documented in the raw data. All animals were acclimated to fasting procedures for 5 days prior to the start of dosing (i.e., for an approximate 6 hour daily fast).

Dosing was not performed on 07-Feb-2003 (day 628), 17-Feb-2003 (day 638) and 18-Feb-2003 (day 639) due to inclement weather and/or site closure. These occurrences had no impact on the study.

Basis of dose selection: Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. High dose of 180 mg/kg/day was lethal and exceeded maximum tolerated dose (MTD). ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, MTD was identified between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to MTD in females. Based on the results of this study, the sponsor initiated the carcinogenicity study at 0 (control feed), 0 (iron supplemented feed), 25, 50 and 100 mg/kg/day. The Exec CAC dated June 5, 2001 (Appendix-1) did not concur with the sponsor's proposed doses of 0, 25, 50 and 100 mg/kg/day. The Committee recommended doses of 15, 30 and 60 mg/kg/day due to concerns for toxicity at higher doses and a concern that the proposed high dose of 100 mg/kg/day is above the MTD.

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These dose levels were subsequently decreased to 15, 30 and 60 mg/kg/day beginning on Day 29, based upon the recommendations of CDER Executive CAC (June 5, 2001, Appendix-1).

Route of administration: Oral (gavage)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Satellite PK: No. Plasma samples were analyzed for toxicokinetic assessment during Weeks 4, 8 (following the dose decrease) and 52. Two animals/sex/group/time point were bled at approximately 0.5, 2, 4, and 7 and 24 hours post dose.

Deviations from original study protocol: During full feeder weight collection on June 17, 2002 (Day 393), full feeder weights for animal nos. 3501- 3526 were inadvertently mis-weighed, as the feed receptacle was inadvertently not tared to zero prior to weighing. As a result of this error, empty feeder weights were not weighed for these animals on June 24, 2002 (Day 400) and food consumption was not calculated. Instead, an entry "NW" (not weighed) was made for these animals. This protocol deviation does not appear to have any significant impact on the study outcome.

Statistical methods:

Mortality data: The survival data was analyzed by using Kaplan-Meier product limit method using SAS/STAT module. The significance of group effects on mortality rates was assessed by applying the logrank test. No Tarone's trend test and no pairwise comparison were conducted, as the group effect was not found to be significant for both sexes.

Tumor data: The trend test was performed for Groups 2, 3, 4 and 5 using Peto's survival adjusted one-sided trend test. According to the recommendations of Lin and Rahman (Lin KK and Rahman MA. 1998. *J Biopharm Stat* 8: 1-15), the dose-related increase in tumor incidence was considered significant if the p-value is ≤ 0.025 for rare tumors and ≤ 0.005 for common tumors (historical incidence of more than 1%). In addition to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. As per the recommendations of Lin and Rahman (1998), the increased tumor rate in a treated group compared to control was considered

significant when the p-value is ≤ 0.05 for a rare tumor, or ≤ 0.01 for a common tumor.

Observations and Times:

Clinical Signs: Clinical signs were observed once daily.

Body Weights: Body weights were recorded once during pretest, and once weekly during Weeks 1 to 14; every four weeks from Weeks 18 to 78; and every 2 weeks thereafter.

Food Consumption: Food consumption was recorded once weekly during Weeks 1 to 14, every fourth week from Weeks 18 to 78 and every 2 weeks thereafter during the dosing period.

Ophthalmoscopy: Ophthalmoscopic examinations (using a Fison Indirect Ophthalmoscope) were conducted on all animals during pretest and on all surviving animals during Weeks 12, 25, 51, 65 and 78. During Weeks 91 and 104 only surviving control and high-dose rats were examined.

Palpable Mass Examination: Palpable mass examinations were conducted monthly for the first 12 months of the study and every two weeks thereafter.

Clinical Pathology: Clinical pathology assessments (hematology and serum chemistry) were conducted on the last 10 surviving animals/sex/group during Weeks 13, 26 and 52.

Plasma and Tissue Iron Analysis: Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) were determined in the serum. In addition, liver (section of right lobe) and kidney (right pole) were collected after exsanguinations from the last 10 surviving rats/sex/group at the terminal sacrifice for the determination of iron content by means of [] following mineralization of the tissue samples.

Histopathology: The tissues specified in the following list (from page 24 of the study report) were examined for histopathology:

Tissue list for collection and/or processing (P)

P	adrenal	P	ovary
P	aorta	P	pancreas
	blood and bone marrow smears	P	parathyroid
P	bone marrow (in bone)	P	pituitary
P	brain	P	preputial gland
P	cecum	P	prostate
P	cervix	P	rectum
P	clitoral gland	P	salivary gland
P	colon	P	sciatic nerve
P	duodenum	P	seminal vesicle
P	epididymis	P	skeletal muscle
P	esophagus	P	skin
P	eye	P	spinal cord
P	femur/tibia	P	spleen
P	Harderian gland	P	sternum
P	heart	P	stomach
P	ileum	P	testis
P	jejunum	P	thymus
P	kidney	P	thyroid
P	lacrimal gland	P	tongue
P	liver	P	trachea
P	lung	P	urinary bladder
P	lymph node – bronchial	P	uterus
P	lymph node – mandibular	P	vagina
P	lymph node – mesenteric	P	macroscopic lesions
P	mammary gland		animal identification
	nasal passage		

Toxicokinetics: Blood was collected from the first 10 surviving animals/sex in each group during Weeks 4, 8 (4 weeks after the dose decrease) and 52. Two surviving animals/sex/group were bled at approximately 0.5, 2, 4, 7 and 24 hours post dose.

Results:

Mortality: No test article-related mortality was observed during the study. There were no apparent differences in mortality rate between the control groups or between the Group 2 control (iron supplemented controls) and test article-treated dose groups. The survival ratios are presented in the following table (from page 27 of the study report).

Table 4-1 Summary of survival ratios with % survival

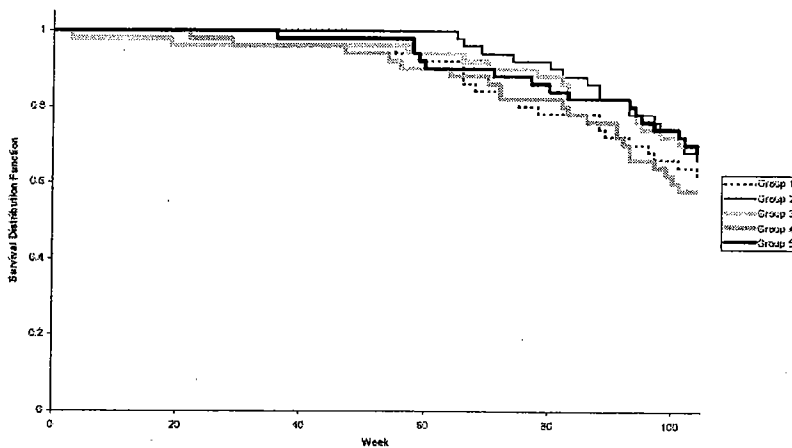
Group:	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Dose:	0	0	25→15	50→30	100→60	0	0	25→15	50→30	100→60
Week										
14	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	49 (98%)	50 (100%)	50 (100%)
26	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	48 (96%)	49 (98%)	50 (100%)
54	50 (100%)	50 (100%)	49 (98%)	49 (98%)	49 (98%)	48 (96%)	50 (100%)	48 (98%)	47 (94%)	49 (98%)
78	47 (94%)	45 (90%)	48 (96%)	46 (92%)	45 (90%)	40 (80%)	46 (92%)	45 (90%)	41 (82%)	43 (86%)
Term	42 (84%)	40 (80%)	38 (76%)	36 (72%)	40 (80%)	31 (62%)	32 (64%)	34 (68%)	29 (58%)	34 (68%)

Dose = mg/kg/day.

The dose decrease for each ICL670 treated group began on day 29.

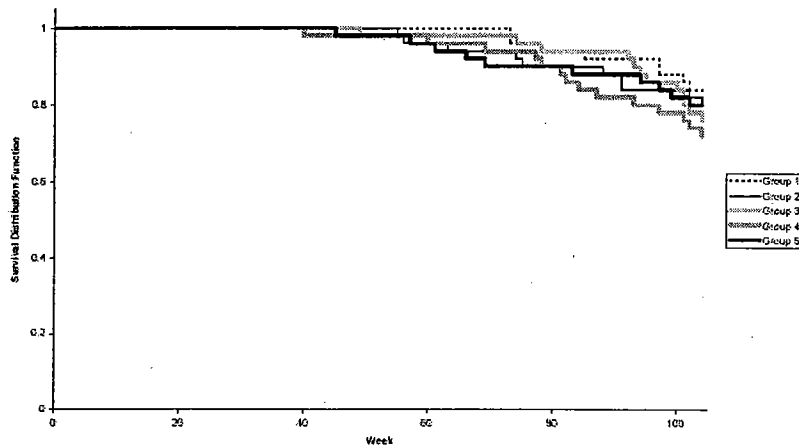
Survival curves are shown in the following figures (from page 46 of the study report).

Figure 2-1 Survival curves for each group of females based on Kaplan-Meier estimates



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Figure 2-2 Survival curves for each group of males based on Kaplan-Meier estimates



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Clinical Signs: Treatment-related clinical signs included slight increases in the incidence of perineal staining (wet staining in the males and wet or dry staining in the females) in both sexes at 100→60 mg/kg/day. In addition, excessive nail growth was observed in all dose groups but was more evident in the iron supplemented controls and test article-treated dose groups. Atypical hairloss (generalized hairloss which progressed to the whole body) and reddened skin was also observed in individual iron supplemented and ICL670-treated animals during the study. The affected animals typically displayed a combination of these signs or all of these clinical signs.

Body Weights: The mean initial and final body weight of control (Control 1) males was 258.4 and 571.9 g, respectively. The mean initial and final body weight of control (Control 1) females was 180.8 and 350.7 g, respectively. The mean initial and final body weight of control (Control 2) males was 256.0 and 549.4 g, respectively. The mean initial and final body weight of control (Control 2) females was 181.8 and 365.9 g, respectively. Statistically significant decreases in mean body weight were observed in the males at high-dose (approximate decreases of 5-6% compared to Group 2 controls) on Days 176-372 and Day 484. Thereafter, the decreases were less than 5% and were negligible by the end of the study. Statistically significant decreases in mean absolute body weight gain were also observed in the males at 100→60 mg/kg/day on the following days: Days 22-64, 85, 148-400, 456-512 and Day 568 (approximate decreases of 9-14% compared to Group 2 controls). The following table shows the body weight data (g).

Day	Males					Females				
	+Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5

1	258.4	256.0	257.2	259.8	254.6	180.8	181.8	182.6	179.9	179.7
15	304.5	299.7	303.1	305.9	294.9	201.5	201.6	201.8	198.8	197.1
29	336.4	329.9	331.2	335.8	318.8	211.6	210.2	211.0	207.5	206.9
92	403.6	391.5	395.2	400.9	379.0	234.8	235.9	236.0	229.1	231.2
176	444.8	431.8	438.5	441.7	411.4*	250.7	253.7	252.3	247.8	247.8
288	488.9	471.3	477.0	484.2	446.1*	270.5	275.9	277.8	271.3	271.6
372	516.1	493.7	502.8	511.9	466.6*	289.0	293.6	293.9	297.9	291.2
540	564.9	536.4	549.5	557.5	509.6	331.2	339.5	328.7	338.6	336.9
722	571.9	549.4	571.8	572.0	539.0	350.7	365.9	356.3	358.6	366.1
Body Weight (% of Control 2)										
722		100%	104%	104%	98.1%		100%	97.4%	98.0%	100%

*: Significantly different from Control (2), $p = 0.05$ with Dunnett's test

+:

Group 1: Control 1 (Standard feed)

Group 2: Control 2 (iron supplemented feed)

Group 3: 25→15 mg/kg/day (iron supplemented feed)

Group 4: 50→30 mg/kg/day (iron supplemented feed)

Group 5: 100→60 mg/kg/day (iron supplemented feed)

Food Consumption: The mean initial and final food consumption in Control 2 males was 22.0 and 21.3 g/animal/day, respectively. The mean initial and final food consumption in Control 2 females was 17.94 and 16.8 g/animal/day, respectively. Food consumption was analyzed comparing Group 2 controls (iron supplemented) to the ICL670-treated dose groups. Slight treatment-related decreases in mean food consumption values were observed in males at 100→60 mg/kg/day. Statistically significant decreases were also observed in males at high-dose on the following days: Day 36-50, 148, 456, 484, 540 and 554. There were no test treatment-related effects on mean food consumption in the females. The following table shows the food consumption (g/animal/day) data.

Day of Study	Males			Females		
	Low-Dose	Mid-Dose	High-Dose	Low-Dose	Mid-Dose	High-Dose
9	21.8	21.7	21.0	18.9	18.0	18.7
15	24.0	23.4	22.9	18.7	18.9	18.6
29	23.2	23.5	22.2	17.9	17.2	17.8
43	22.9	22.8	22.0	17.5	16.8	17.4
57	22.4	22.8	21.9	17.3	17.1	16.9
78	22.7	22.2	22.0	18.0	17.4	17.7
92	21.2	21.5	21.3	17.0	17.0	16.9

Ophthalmology: Ophthalmoscopic examinations performed during the course of the study did not reveal any ocular changes attributable to treatment with ICL670.

Palpable Mass: There was no apparent increase in the incidence of palpable masses observed during the course of the study or prior to scheduled necropsy. A

summary of the palpable mass incidence at the last scheduled necropsy is presented in the following table (page 29 of the study report).

Table 4-2 Palpable mass incidence at final palpable mass exam (day 723)

Group Dose (mg/kg/day)	Incidence (% incidence)	
	Males	Females
1 0 (standard diet)	9/42 (21.4%)	9/32 (28.1%)
2 0 (iron supplemented diet)	6/41 (14.6%)	8/34 (23.5%)
3 25→15 mg/kg/day (iron supplemented diet)	3/39 (7.7%)	9/35 (25.7%)
4 50→30 mg/kg/day (iron supplemented diet)	5/36 (13.9%)	2/29 (6.9%)
5 100→60 mg/kg/day (iron supplemented diet)	6/40 (15%)	5/34 (14.7%)

Hematology: No treatment-related changes were observed.

Serum Iron Analysis: Serum iron analyses revealed slight increases in serum iron concentrations in the iron supplemented controls (both sexes at Week 13), in males at 100→60 mg/kg/day (Weeks 13, 26 and 52) and in the females at all dose levels (Weeks 13, 26 and 52). Unsaturated iron binding capacity (UIBC) was decreased minimally in iron-supplemented control rats (both sexes), in Group 5 males and Groups 3, 4, and 5 females at all time points, and increased marginally in Group 3 males at all time points. However, the total iron binding capacity (TIBC) was not altered.

Tissue Iron Analysis: Dose-related decreases in mean liver and kidney iron content were evident in males at all doses when compared to the iron supplemented controls (approximately 57-73% reduction in the liver and approximately 40-70% reduction in the kidney) and when compared to control males fed the standard rodent diet (approximately 40-63% reduction in the liver and 24-61% reduction in the kidney). In addition, dose-related decreases in mean liver and kidney iron content were also observed in females at all doses when compared to the iron supplemented controls (approximately 34-72% reduction in the liver and approximately 38-74% reduction in the kidney) and when compared to control females fed the standard rodent diet (approximately 28-69% reduction in the liver and approximately 18-66% reduction in the kidney). Iron supplementation of the controls increased the tissue iron content by 28-41% in males and by 9-32% in the females.

Macroscopic Examinations: There were no treatment-related macroscopic findings.

Histopathology:

Non-Neoplastic: There appears to be no significant treatment-related findings. However, histopathological changes were seen in different tissues, which do not appear to be treatment-related either in the absence of a dose-response or presence of these changes in the control animals also. The following table shows some of the histopathological observations.

Tissue Findings	Males					Females				
	Dose (mg/kg/day)					Dose (mg/kg/day)				
	0 (n*=50)	0 (n=50)	25→15 (n=50)	50→30 (n=50)	100→60 (n=50)	0 (n*=50)	0 (n=50)	25→15 (n=50)	50→30 (n=50)	100→60 (n=50)
HEART										
Inflammation, acute								1		
Fibrosis, pericardium									1	
Periarteritis		1		1						
Mineralization, aorta	1								1	
Hyperplasia, endocardium										1
SALIVARY GLAND										
Inflammation	1		1		2		1	1		
Metaplasia, squamous			1							
Atrophy, acinal, focal								1		1
Hemorrhage									1	
STOMACH										
Edema, non-glandular		1	1	1		1	4	2	2	
Erosion, non-glandular		1		2	1		1	4		1
Hyperplasia, mucosa, glandular, focal						1				1
Inflammation, nonglandular		1	1	1		1	2	1	1	
Ulcer, nonglandular				1			2	2	2	
LIVER										
Hyperplasia, bile duct	7	5	10	8	7	15	11	10	11	11
Inflammation	5	3	8	8	7	3	3	5	8	4
Necrosis, focal	1	1	1					1	1	
Necrosis, centrilobular			1							1
Extramedullary hematopoiesis	2	3	2	3	2	3	3	3	2	2
Necrosis								1		

Fibrosis, periportal									1	
ADRENAL										
Atrophy, cortex					1			2		
Hypertrophy, cortex, focal			1	2		1		3	1	1
Infiltrate, lymphocyte					1					
Necrosis, cortex							1	1	2	2
PITUITARY										
Hemorrhage				1	1					
THYROID										
Inflammation								1	1	1
KIDNEY										
Hyperplasia, epithelium, pelvis					1					
Inflammation	1	1	5	3	4	1		3	4	2
Necrosis, papillary	1				1			1		1
Necrosis, tubular			1	2	2	4	4	4	4	4
Pyelonephritis	1							1	2	1
SEMINAL VESICLE										
Inflammation	1		2	1	3					
TESTIS										
Atrophy, somniferous tubule	7	9	9	10	8					
URINARY BLADDER										
Hyperplasia, transitional	1				2			1	1	1
THYMUS										
Hyperplasia, focal		1	1	2		3		2		1
SPLEEN										
Necrosis, focal										1
Hyperplasia, lymphoid										2

*: n = Number of animals examined

Neoplastic: In this study, one-sided trend test was performed for comparisons across Groups 2, 3, 4 and 5 using Peto's survival adjusted trend test. For males and females, the one-sided overall trend tests were not found to be significant in any case ($p > 0.05$). In addition to the overall trend test, pairwise comparisons were made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The increased incidence of tumors in the treated groups 3, 4 and 5 was not considered significant for all neoplastic lesions, as their p-values were > 0.05 . The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. However, when comparing the

tumor incidence in Group 1 to Group 2, the differences were found to be significant ($p = 0.0344$) for combined c-cell adenomas or carcinomas in the thyroid of males if this tumor is classified as a rare tumor. Overall, there was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates. It is to be mentioned here that the sponsor provided limited historical control data for this testing facility from three different studies conducted during the period of 1999-2003. The tumor incidences are shown in the following table (data extracted from page 1003-1035 of sponsor's report).

Organ/Tumor	Contro l 1 (n = 50)	Contro l 2 (n = 50)	25→15 mg/kg/da y (n = 50)	50→30 mg/kg/da y (n = 50)	100→60 mg/kg/da y (n = 50)	p- Value *	Historical Control Incidence s
FEMALES							
Adrenals							
Cortical adenoma	2 (0%)	1 (2%)	0 (0%)	1 (2%)	1 (2%)	0.4446	1.76%
Pheochromocytoma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.62%
Brain							
Granular cell tumor	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2595	0.6%
Clitoral Gland							
Squamous cell carcinoma	0 (0%)	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/48 (2.1%)	0.2558	NA
Liver							
Hepatocellular carcinoma	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0.9380	0.6%
Lung							
Adenoma, bronchioloalveolar	0 (0%)	1 (2%)	1 (2%)	0/49 (0%)	1/49 (2%)	0.5378	NA
Mammary Gland							
Adenocarcinoma	6 (12%)	3 (6%)	1 (2%)	2 (4%)	0 (0%)	0.9313	12.3%
Adenolipoma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Adenoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2457	2.9%
Fibroadenoma	4 (8%)	9 (18%)	8 (16%)	3 (6%)	7 (14%)	0.7502	18.2%
Fibroma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Muscle							
Rhabdomyosarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7403	NA
Ovary							
Adenocarcinoma	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Luteal cell tumor	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2593	NA
Thecal cell tumor	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	1.2%
Pancreas							
Islet cell adenoma or carcinoma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.0%
Parathyroid							
Adenoma	0/46 (0%)	1/47 (2.1%)	2/47 (4.2%)	0/47 (0%)	0/48 (0%)	0.9390	NA
Pituitary							
Adenoma or carcinoma, pars	28 (56%)	29 (58%)	24 (48%)	28 (56%)	28 (56%)	0.4263	NA

distalis							
Adenoma, pars intermedia	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA
Carcinoma, pars intermedia	0 (0%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	0.7989	NA
Reproductive Tract							
Polyp, Endometrial stromal polyp or sarcoma	4 (8%)	8 (16%)	8 (16%)	7 (14%)	3 (6%)	0.9366	NA
Skin							
Basal cell tumor	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	0.0%
Fibroma or fibrosarcoma	1 (2%)	0 (0%)	1 (2%)	0 (0%)	2 (4%)	0.1091	NA
Fibrosarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	1 (2%)	0.3126	NA
Stomach							
Sarcoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2585	NA
Squamous cell carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2593	NA
Systemic							
Histiocytic sarcoma	0 (0%)	0 (0%)	3 (6%)	0 (0%)	0 (0%)	0.8352	NA
Thymus							
Thymoma	2 (4%)	5 (10%)	5 (10%)	6 (12%)	7 (14%)	0.2550	11.8%
Thyroid							
C-cell adenoma or carcinoma	5/49 (10.2%)	13 (26%)	7 (14%)	7 (14%)	5 (10%)	0.9684	NA
Follicular adenoma or adenocarcinoma	0/49 (0%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)	0.8314	NA
Uterus							
Adenocarcinoma	0 (0%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)	0.5636	3.5%
Choriocarcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2500	NA
Endometrial stromal polyp or sarcoma	4 (8%)	6 (12%)	7 (14%)	7 (14%)	3 (6%)	0.8408	1.76%
Vagina							
Granular cell tumor	2 (4%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	1.0000	10.6%
Polyp	0 (0%)	2 (4%)	1 (2%)	0 (0%)	0 (0%)	0.9743	NA
Whole Body							
Hemangiosarcoma	1 (2%)	2 (4%)	1 (2%)	0 (0%)	0 (0%)	0.9851	NA
Leiomyosarcoma	3 (6%)	2 (4%)	0 (0%)	1 (2%)	1 (2%)	0.7016	NA
Lipoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7481	NA
Nerve sheath tumor	1 (2%)	0 (0%)	1 (2%)	0 (0%)	2 (4%)	0.1144	NA
MALE							
Adrenal							
Cortical adenoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (4%)	0.0635	1.18%
Pheochromocytoma	3 (6%)	2 (4%)	1 (2%)	1 (2%)	0 (0%)	0.9380	2.9%
Brain							
Astrocytoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2597	1.2%
Granular cell tumor	0 (0%)	0 (0%)	2 (4%)	0 (0%)	1 (2%)	0.5000	1.17%
Epididymides							

Mesothelioma	0 (0%)	0 (0%)	2 (4%)	2 (4%)	0 (0%)	0.6807	NA
Kidney							
Adenoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2597	NA
Transitional cell carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4913	NA
Liver							
Hepatocellular adenoma or carcinoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)	2 (4%)	0.2531	NA
Hepatocellular carcinoma	0 (0%)	1 (2%)	0 (0%)	2 (4%)	2 (4%)	0.1795	1.8%
Lung							
Adenoma, bronchioloalveolar	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2105	NA
Muscle							
Fibrosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4944	NA
Pancreas							
Islet cell adenoma or carcinoma	2 (4%)	0 (0%)	3 (6%)	3 (6%)	2 (4%)	0.2749	NA
Parathyroid							
Adenoma	2/49 (4.1%)	1 (2%)	1/48 (2.1%)	1/49 (2.0%)	0/48 (0%)	0.8312	1.17%
Pituitary							
Adenoma or carcinoma, pars distalis	17 (34%)	15/49 (30.6%)	19 (38%)	15 (30%)	21 (42%)	0.1706	NA
Adenoma, pars intermedia	1 (0%)	1/49 (2.0%)	2 (4%)	0 (0%)	0 (0%)	0.9278	NA
Carcinoma, pars distalis	0 (0%)	0/49 (0%)	0 (0%)	0 (0%)	1 (2%)	0.2575	0.6%
Prostate							
Adenocarcinoma	0 (0%)	1 (2%)	0 (0%)	1 (2%)	0 (0%)	0.8050	NA
Rectum							
Sarcoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0.7403	NA
Salivary Gland							
Adenocarcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4935	NA
Skin							
Fibroma or fibrosarcoma	1 (2%)	2 (4%)	0 (0%)	0 (0%)	2 (4%)	0.4172	NA
Testis							
Interstitial cell tumor	2 (4%)	3 (6%)	1 (2%)	1 (2%)	0 (0%)	0.9787	1.76%
Thymus							
Thymoma	2/49 (4.1%)	2 (4%)	0/49 (0%)	1/48 (2.1%)	3 (6%)	0.2082	3.5%
Thyroid							
C-cell adenoma or carcinoma	3 (6%)	10 (20%)	7 (14%)	10 (20%)	9 (18%)	0.4804	NA
Whole Body							
Hemangiosarcoma	0 (0%)	1 (2%)	1 (2%)	5 (10%)	0 (0%)	0.6704	NA
Leiomyosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0.4935	NA

Lipoma	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1.0000	NA

*: p-values are from one-sided trend tests across Groups 2 to 5.

Trend Test: $p \leq 0.025$ (Significant for rare tumors); $p \leq 0.005$ (Significant for common tumors)

Pairwise Comparison: $p \leq 0.05$ (Significant for rare tumors); $p \leq 0.01$ (Significant for common tumors)

Toxicokinetics: Initially, at Week 4, the exposure [$AUC_{(0-24h)}$] appeared to increase over-proportionally with the dose in both sexes, between doses of 25 to 100 mg/kg/day. There was an apparent gender difference in exposure at the beginning of the study. Exposure in females was found to be higher at the mid- and high-dose when compared to male rats. The exposure increased approximately two-fold between Week 8 and 52 at the same dose, based on $AUC_{(0-24h)}/\text{dose}$. After 8 and 52 weeks of treatment, the exposure increased over-proportionally between doses of 15 to 60 mg/kg/day, in both sexes. After 52 weeks of treatment, there was no apparent gender differences in exposure to ICL670 at the low- and mid-dose (15 and 30 mg/kg/day); however, the exposure was found to be higher in males than in females at the high dose (60 mg/kg/day). The mean TK parameters are shown in the following table (from page 31 of the study report).

Table 4-3 Mean toxicokinetic parameters of total ICL670 in rats

Doses (mg/kg/day)	Sampling period (week)	C_{max} ($\mu\text{mol/L}$)		$AUC_{(0-24h)}$ ($\mu\text{mol.h/L}$)		$AUC_{(0-24h)}/\text{dose}$ ($\mu\text{mol.h/L}/(\text{mg/kg/day})$)	
		male	female	male	female	male	female
25	4	23.7	18.3	71.0	30.7	2.84	1.23
15*	8	5.13	13.6	10.9	20.4	0.724	1.36
15*	52	12.9	13.2	32.8	42.7	2.19	2.85
50	4	31.7	44.5	89.9	165	1.80	3.30
30*	8	12.9	35.5	43.3	72.2	1.44	2.41
30*	52	41.6	46.2	123	135	4.11	4.52
100	4	189	310	607	921	6.07	9.21
60*	8	81.5	61.7	217	115	3.61	1.91
60*	52	178	76.9	543	387	9.05	6.45

*Doses were reduced on day 29.

Summary Study Findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The study was considered adequate. The test model (Wistar Hannover rats) was also considered appropriate. Doses [0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron)] were used as per

ExecCAC recommendations. Doses were reduced on Day 29 as per the recommendations of the Executive CAC. Overall, the conduct of the study was considered appropriate and acceptable. There was adequate number of animals at the end of the study in each group for statistical considerations. It is to be mentioned here that the sponsor submitted limited historical control data from this testing facility from three studies conducted during the period of 1999-2003.

Evaluation of tumor findings: In this study, the trend test was performed for Groups 2, 3, 4 and 5 using Peto's survival adjusted trend test. For males and females, the one-sided overall trend tests were not found to be significant in any case ($p > 0.05$). According to the recommendations of Lin and Rahman (Lin KK and Rahman MA. 1998. *J Biopharm Stat* 8: 1-15), the dose-related increase in tumor incidence was considered significant if the p-value is ≤ 0.025 for rare tumors and ≤ 0.005 for common tumors (historical incidence of more than 1%). Using these criteria, the trend test was found to be non-significant, as all the p-values were > 0.025 . In addition to the overall trend test, pairwise comparisons were also made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the control Group 2. The tumor incidences in Group 1 were also compared to Group 2 using Peto's two-sided trend test. For each pair-wise comparison, only the two considered groups were used in the analysis. In both sexes, there was no tumor or tumor combinations for which p-value was found to be ≤ 0.05 . As per the recommendations of Lin and Rahman (1998), the increased tumor rate in a treated group compared to control was considered significant when the p-value is ≤ 0.05 for a rare tumor, or ≤ 0.01 for a common tumor. Using these criteria, the increased incidence of tumors in the treated Groups 3, 4 and 5 was not considered significant for all neoplastic lesions, as their p-values were > 0.05 . However, when comparing the tumor incidence in Group 1 to Group 2, the differences were found to be significant ($p = 0.0344$) for combined c-cell adenomas or carcinomas in the thyroid of males if this tumor is classified as a rare tumor. Overall, there was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

Carcinogenicity Summary: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel, to 5 groups of Wistar Hannover rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). Doses were decreased on Day 29 of the study based upon the recommendations of ExecCAC. Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. No test article-related mortality or moribundity was evident upon histopathological assessment. At study termination, 72-84% survival was observed in the males and 58-68% survival was observed in the females. There were no statistical differences in survival between the control groups and ICL670-treated dose groups. There were no test article-related effects noted during

palpable mass examination. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

Carcinogenicity Conclusions: In conclusion, based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks was not appeared to be carcinogenic at doses up to 60 mg/kg/day in either sex.

Recommendations for further analysis: None.

Labeling Recommendations: None

Addendum/Appendix Listing:

Appendix-1: Exec CAC meeting minutes dated June 5, 2001, page: 282

Appendix-2: List of neoplastic and non-neoplastic lesions, page: 286

Dose-Ranging Study Report: None

CAC Report: None

Alternative Study Protocols and CAC Report: None

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Study Title: 26-Week Oral (Gavage) Carcinogenicity Study in p53 (+/-) Transgenic Mice**Key Study Findings:**

Treatment-related moribundity/mortality occurred in one p53 heterozygous female at 300 mg/kg/day receiving the iron supplemented diet and one p53 heterozygous female at 100→60 mg/kg/day receiving the standard rodent diet. Nine other p53 mice (4 females, supplemented with dietary iron at 300 mg/kg/day and 5 females, without dietary iron supplementation at 100 mg/kg/day) were also considered test article-related. One test article-related moribundity was also observed in a high-dose wild type male at 200 mg/kg/day with dietary iron.

Test article-related decreases in mean body weight were observed in p53 heterozygous mice supplemented with iron at 200 mg/kg/day (males) and 300 mg/kg/day (females), in p53 heterozygous mice receiving standard feed in the males at 30 mg/kg/day and in the females at 100→60 mg/kg/day and in wild type mice supplemented with iron in males at 200 mg/kg/day and females at 300 mg/kg/day.

Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female mouse at 300 mg/kg/day.

There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation.

The major effects observed with *p*-cresidine, the positive control, included proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%).

Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were predominantly observed in non-supplemented mice.

Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to

200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Study Number: 0270117

Volume #, and Page #: Page 1 of Report 0270117

Conducting Laboratory and Location: Novartis Pharmaceuticals Inc., East Hanover, NJ

Date of Study Initiation: October 7, 2002

Date of Study Completion: May 24, 2004

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, ζ 1

CAC Concurrence: Yes. Exec CAC meeting minutes dated August 21, 2001 (Appendix-3), September 17, 2001 (Appendix-4) and November 12, 2002 (Appendix-5) are attached.

Study Type: Transgenic assay

Species/Strain:

B6.129-Trp53^{tm1} N5 mouse (p53 heterozygous) or C57BL/6/TacBR-[KO]p53 N5 mouse (p53 heterozygous)

B6.129-Trp53^{tm1} N5 mouse (p53 wild-type) or C57BL/6/TacBR-[KO]p53 N5 mouse (p53 wild-type)

Number/Sex/Group; Age at Start of Study: 25/sex/group; Age: 11 weeks old

Body Weight: Males: 22 to 32 g; Females: 17 to 25 g

Animal Housing: Animals were housed singly in solid bottom cage.

Formulation/Vehicle: Suspension in 0.5% (w/v) Klucel (type HF), NF

Drug Stability/Homogeneity: Based on stability data, ICL670 in 0.5% Klucel, 0.75 mg/mL to 100 mg/mL, was found to be stable for use for at least ζ 7 at 6°C and at least ζ stirring at room temperature. Aqueous 0.5% Klucel was found to be suitable for use for at least ζ 7 stirring at room temperature and at least ζ 7 stored at 6°C. The uniformity of dosing formulations was also confirmed. Samples of the formulations for Weeks 1, 8, 13, and 26 were tested, and the concentrations were found to be ζ of target concentrations. Six control samples were also tested, and no ICL670 was detected.

Methods:

Doses: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two

additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The dosing volume for all dose groups was 10 ml/kg. The doses for the current study were selected as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 17, 2002, and November 12, 2002, Appendix-3, 4, and 5, respectively). Doses are shown below.

p53 Heterozygous Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)
- Group 4: 100 mg/kg/day (feed + iron)
- Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)
- Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)
- Group 7: p-Cresidine (400 mg/kg/day, oral)
- Group 8: 0 mg/kg/day (female, standard feed)
- Group 9: 30 mg/kg/day (female, standard feed)

Wild Type Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

The following table presents the study design (from page 19 of sponsor's report).

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Table 3-2 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (heterozygous mice): [C] protocol number 0270117					
Group	Number/sex	Animal Numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1 Control	25 2 toxicokinetic	1001-25	1501-25	0 (feed with iron)	0 (feed with iron)
		1026-27	1526-27		
2 Control	25 2 toxicokinetic	2001-25	2501-25	0 (standard feed)	0 (standard feed)
		2026-27	2526-27		
3	25 6 toxicokinetic	3001-25	3501-25	20 (feed with iron)	30 (feed with iron)
		3026-31	3526-31		
4	25 6 toxicokinetic	4001-25	4501-25	100 (feed with iron)	100 (feed with iron)
		4026-31	4526-31		
5	25 6 toxicokinetic	5001-25	5501-25	200 (feed with iron)	300 (feed with iron)
		5026-31	5526-31		
6	25 6 toxicokinetic	6001-25	6501-25	30 (standard feed)	100→60* (standard feed)
		6026-31	6526-31		
7 Positive control	25	7001-25	7501-25	400 p-cresidine (non-supplemented feed)	
8** Control	25 2 toxicokinetic		8501-25	0 (standard feed)	
			8526-27		
9**	25 6 toxicokinetic		9501-25	30 (standard feed)	
			9526-31		

*Dose decrease effective 25-Nov-2002 (day 50) based on the early sacrifice/death of female mice at 100 mg/kg/day.

**Additional female dose groups were added because of early sacrifice/death in the group 6 female mice.

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. Animals in groups 1-7 were acclimated to fasting procedures for 2 weeks prior to the start of dosing. Animals in groups 8-9 were acclimated to fasting procedures for approximately 1 week prior to dosing.

In order to facilitate on-line data collection, p53 heterozygous mice were assigned to — protocol number 0270117 (groups 1-7) or — protocol number 0270117f (groups 8 and 9). The wild type mice were assigned to — protocol number 0270117w.

Table 3-3 Study design, animal allocation and test article doses

B6.129-Trp53 ^{tm1} N5 (wild type mice): [C] protocol number 0270117w					
Group	Number/sex	Animal numbers		Dose ICL670 (mg/kg/day)	
		Males	Females	Males	Females
1	25	1001-25	1501-25	0	0
Control	2 toxicokinetic	1026-27	1526-27	(feed with iron)	(feed with iron)
2	25	2001-25	2501-25	0	0
Control	2 toxicokinetic	2026-27	2526-27	(standard feed)	(standard feed)
3	25	3001-25	3501-25	200	300
	6 toxicokinetic	3026-31	3526-31	(feed with iron)	(feed with iron)

Dosing was not performed on 07-Feb-2003, 17-Feb-2003 and 18-Feb-2003 due to inclement weather. These occurrences had no impact on the study.

Animals were fasted daily (approximately 4 hours) prior to dosing in order to facilitate absorption of ICL670. Food was re-presented to the animals approximately 2 hours from the midpoint time of daily dosing. Due to inclement weather on 05-Dec-2002 and 03-Jan-2003, animals were fasted for about 2.5 hours and food was re-presented to the animals about 1 hour from the midpoint of dosing. Other changes to the feeding schedule were documented in the raw data. All animals were acclimated to fasting procedures for 2 weeks prior to the start of dosing.

Basis of dose selection: Doses for this study (0170121) were based on results of a 4-week oral (gavage) range finding toxicity study in C57BL/6 mice. In this dose ranging study, ICL670 was administered in aqueous 0.5% Klucel to 4 groups of mice fed an iron supplemented diet at doses of 0, 100, 300, and 600→450 mg/kg/day. ICL670 was also administered to 3 groups of mice fed a standard diet at doses of 0, 100 and 600 mg/kg/day. The doses for the current study were selected based on the CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 17, 2002, and November 12, 2002, Appendix-3, 4, and 5, respectively).

Route of administration: Oral (gavage)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Satellite PK: Yes. Blood samples were collected from satellite animals at approximately 0.5, 2 and 6 hours postdose during Week 23 to determine the toxicokinetic (TK) profile of ICL670.

Deviations from original study protocol: On January 18, 2003 (Day 31), the Group 8 female toxicokinetic control dose group (animal nos. 8526 and 8527)

were inadvertently dosed with the 3 mg/ml formulation intended for the Group 9 study animals (30 mg/kg/day). This dosing error did not appear to have any significant impact on the study or the TK results, since no ICL670 was detected during the Week 23 TK sampling.

Statistical methods: In-life data (body weight, food consumption, clinical pathology and organ weights): Means and standard deviations for body weight and food consumption determinations were calculated and an Analysis of Variance (ANOVA) was performed. The ANOVA was followed by a Bartlett's test for homogeneity of variances. When the variances were homogeneous, Dunnett's t-test was employed to determine the statistical significance between control and treated groups. When the variances were not homogeneous, a modified t-test was used to determine which groups were statistically different from the controls. Means and standard deviations were calculated for the clinical pathology and organ weight data. The sponsor did not perform statistical analysis of tumor incidences.

Observations and Times:

Clinical Signs: Clinical signs were observed once daily.

Body Weights: Body weights were recorded once during pretest, and once weekly during Weeks 1 to 14; every two weeks from Weeks 16 to 26.

Food Consumption: Food consumption was recorded once weekly during Weeks 1 to 14, every two weeks from Weeks 16 to 26.

Ophthalmoscopy: Ophthalmoscopic examinations (using a Fison Indirect Ophthalmoscope) were conducted on all animals during pretest and on all surviving animals during Weeks 4, 15, and 26.

Palpable Mass Examination: Palpable mass examinations were conducted on all main study animals beginning Week 2 and every two weeks thereafter.

Clinical Pathology: Clinical pathology assessments (hematology and serum chemistry) were conducted on the last 10 surviving animals/sex/group at scheduled necropsy.

Plasma and Tissue Iron Analysis: Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) were determined in the serum. In addition, liver (section of right lobe) and kidney (right pole) were collected after exsanguination from the last 10 surviving animals/sex/group at the terminal

sacrifice for the determination of iron content by means of [] following mineralization of the tissue samples.

Organ Weights: The following organs were weighed (marked with “W” in the table below) from all animals: brain, heart, kidney, liver, spleen, testis and thymus.

Histopathology: The tissues specified in the following list (from page 28 of the study report) were examined for histopathology:

Tissue list: for Collection and/or Processing (P)

	P	adrenal			nasal passage
	P	aorta		P	ovary
	P	blood and bone marrow smears		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
W	P	brain		P	pituitary
	P	cecum		P	preputial gland
	P	cervix		P	prostate
	P	clitoral gland		P	rectum
	P	colon		P	salivary gland
	P	duodenum		P	sciatic nerve
	P	epididymis		P	seminal vesicle
	P	esophagus		P	skeletal muscle
	P	eye		P	skin
	P	femur/tibia		P	spinal cord
	P	gallbladder	W	P	spleen
	P	harderian gland		P	sternum
W	P	heart		P	stomach
	P	ileum	W	P	testis
	P	jejunum	W	P	thymus
W	P	kidney		P	thyroid
	P	lacrimal gland		P	tongue
W	P	liver		P	trachea
	P	lung		P	urinary bladder
	P	lymph node – bronchial		P	uterus
	P	lymph node – mandibular		P	vagina
	P	lymph node – mesenteric		P	macroscopic lesions
	P	mammary gland			animal identification (right ear, left ear or tail)

Toxicokinetics: Blood was collected from all TK animals during Week 23. For the control groups, surviving TK animals were bled at approximately 2 hours post dose. For ICL670-treated animals, surviving animals were bled at approximately 0.5, 2 and 6 hours postdose.

Results:

Mortality:

Heterozygous mice fed an iron-supplemented diet: Test article-related mortality was observed in one high dose female at 300 mg/kg/day supplemented with iron (animal no. 5518 on Day 124). The cause of death was marked erosion of the glandular stomach. In addition, four additional p53 heterozygous mice that died or were sacrificed early (animal nos. 5501, 5504, 5505 and 5509 treated at 300 mg/kg/day, all supplemented with iron) between Days 56 and 170 were also considered test article-related. The cause of death could not be confirmed upon histopathological assessment; however a relationship to the treatment was not ruled out. In animal nos. 5501, 5504 and 5509 relevant clinical signs which were observed before sacrifice included severe decreased locomotor activity, cold to touch, hunched posture, ptosis, reduced feces and labored respiration. The remaining heterozygous mice supplemented with iron that died or were sacrificed early were considered to be due to gavage accidents (animal nos. 1505, 4007, 4016, 5519 and 5524), lymphoma (animal nos. 3021, 3023 and 3505), osteosarcoma (animal nos. 1506, 5516), mammary adenocarcinoma (animal no. 4512) or arteritis (animal no. 4522). One female (animal no. 4502 treated at 100 mg/kg/day) was sacrificed on Day 183 due to an apparent injury which caused hindlimb paralysis. A gross lesion on the leg was collected at necropsy, however there were no microscopic findings. Although the cause of death of this animal could not be determined; however, it considered to be due to injury and therefore was considered treatment-related. The following table (from page 32 of the study report) shows the summary of early death/sacrifice in heterozygous mice fed on iron-supplemented diet.

For heterozygous mice supplemented with iron:

Table 4-1 Summary of early death/sacrifice (heterozygous mice with iron)

	Males				Females			
		Dose (mg/kg/day)				Dose (mg/kg/day)		
Group:	1	3	4	5	1	3	4	5
	(0)	(20)	(100)	(200)	(0)	(30)	(100)	(300)
Week								
26	0	2	2	0	2	1	3	8*

*Five of the eight early deaths/sacrifices were considered test article-related.

For heterozygous mice fed a standard diet: Treatment-related mortality was observed in one female at 100 mg/kg/day (animal no. 6525 on Day 22). Relevant clinical signs preceding sacrifice included cold to touch, thin appearance,

moderate decreases in locomotor activity and moderate ataxia. The cause of death was considered to be stomach ulceration. In addition, five other females that died or were sacrificed early (Animal nos. 6504, 6505, 6514, 6520 and 6523) between Days 36 and 42 of the study were also considered treatment-related. However, the cause of death was not evident upon histopathological examinations. Clinical signs for these five animals included decreased locomotor activity, dehydration, thin appearance, hunched posture, muscle tremors and/ or labored respiration prior to death or sacrifice. In addition, one male (animal no. 6002) treated at 30 mg/kg/day was sacrificed moribund on Day 157. The cause of death was considered to be due to inflammation of the secondary sex organs surrounding the lower urinary tract and was not considered treatment-related. One control female (animal no. 2518) was sacrificed on Day 183. The cause of death was arteritis. The following table (from page 32 of the study report) shows the mortality in heterozygous mice fed with standard diet.

For heterozygous mice fed a standard diet:

Table 4-2 Summary of early death/sacrifice (heterozygous mice, no iron)

	Males			Females		
	Dose (mg/kg/day)			Dose (mg/kg/day)		
Group:	2 (0)	6 (30)	7 (400 p-cresidine)	2 (0)	6 (100→60)	7 (400 p-cresidine)
Week						
26	0	1	2	1	6*	0

*All of the six early deaths/sacrifices were considered test article-related.

p-Cresidine mice: Two males died during the treatment phase. Animal no. 7009 was found dead on Day 105 and animal no. 7015 was found dead on Day 175. These animals exhibited slightly decreased locomotor activity (7015) and animal 7009 had a visible swelling/mass. The cause of death in these animals was considered to be due to skin carcinoma (animal no. 7009) or urinary bladder carcinoma (animal no. 7015). The above table (from page 33 of the study report) shows summary of mortality in p-cresidine animals.

Heterozygous mice fed on a standard diet (Additional Groups 8 and 9): Animal no. 9503 was sacrificed on Day 19. Based on the macroscopic and microscopic findings, the cause of death was considered to be due to gavage accident. The following table (from page 33 of the study report) shows the mortality in Groups 8 and 9.

Table 4-3 Summary of early sacrifice/death (heterozygous mice, no iron)

Females		
Group:	8	9
Dose:	0 mg/kg/day	30 mg/kg/day
Week		
26	0	1

Wild type mouse fed on an iron-supplemented diet: Animal no. 3021 treated at 200 mg/kg/day was sacrificed moribund on Day 114. The cause of death was considered to be due to stomach and kidney lesions which were attributable to treatment. This animal also had lesions in the lung which suggested that misgavage may also have contributed to the moribundity of this animal. One female (animal no. 3514 treated at 300 mg/kg/day) was sacrificed for humane reasons on Day 85. The cause of death was considered as arteritis. This animal exhibited decreased locomotor activity, hunched posture, ptosis, reduced feces and piloerection of the fur and the female exhibited head tilt, circular movements, impaired righting reflex, ataxia and a thin appearance. The following table (from page 34 of the study report) shows the mortality in wild type mice fed on iron-supplemented diet.

For the wild type mouse supplemented with iron:

Table 4-4 Summary of early deaths/sacrifices (wild type with iron)

	Males			Females		
	Dose (mg/kg/day)			Dose (mg/kg/day)		
Group:	1	2	3	1	2	3
	(0)	(0)	(200)	(0)	(0)	(300)
Week						
26	0	0	1*	0	0	1

*Moribund sacrifice considered test article-related.

The following table shows the summary of mortality in Groups 1-9 in males and females.

MALE								
GROUPS*								
1	2	3	4	5	6	7		
0	0	2	2	0	1	2		
FEMALES								
GROUPS								
1	2	3	4	5	6	7	8	9
2	1	1	3	8**	6**	0	0	1

** : Deaths considered treatment-related

.*:

p53 Heterozygous Mice

- Group 1: Control 1 (feed + iron)
 - Group 2: Control 2 (standard feed)
 - Group 3: 20 mg/kg/day (male, feed + iron), 30 mg/kg/day (female, feed + iron)
 - Group 4: 100 mg/kg/day (feed + iron)
 - Group 5: 200 mg/kg/day (male, feed + iron), 300 mg/kg/day (female, feed + iron)
 - Group 6: 30 mg/kg/day (male, standard feed), 100→60 mg/kg/day (female, standard feed)
 - Group 7: p-Cresidine (400 mg/kg/day, oral)
 - Group 8: 0 mg/kg/day (female, standard feed)
 - Group 9: 30 mg/kg/day (female, standard feed)
- Wild Type Mice

- Group 1: Control 1 (feed + iron)
- Group 2: Control 2 (standard feed)
- Group 3: 200 mg/kg/day (male, feed with iron), 300 mg/kg/day (feed with iron)

Clinical Signs: Treatment-related clinical signs observed in animals that were died or sacrificed due to moribund conditions generally included severely decreased locomotor activity, cold to touch, hunched posture, ptosis, reduced feces, labored respiration, dehydration, thin appearance and/or muscle tremors. For the *p*-cresidine mice, all animals exhibited slightly decreased locomotor activity. Additional sings included ptosis, wet stains on fur and a thin appearance.

Body Weights:

For heterozygous mice supplemented with iron (Groups 1, 3, 4 and 5):

The mean initial and final body weights of control (Group 1) males were 24.2 and 37.8 g, respectively. The mean initial and final body weights of control (Group 1) females were 19.1 and 30.9 g, respectively. There were no test article-related effects on mean body weight in either sex at doses up to 100 mg/kg/day. Test article-related decreases in mean body weight were observed at 200 mg/kg/day in males (6-7%) and 300 mg/kg/day in females (6-8%). Body weights in Groups 1, 3, 4 and 5 are shown in the following table.

Day	Males (Iron supplemented)				Females (Iron supplemented)			
	Group 1	Group 3	Group 4	Group 5	Group 1	Group 3	Group 4	Group 5
-3	24.2	24.3	24.3	24.5	19.1	18.9	19.1	18.7
15	28.2	28.4	28.2	28.2	23.2	22.5	22.4	22.8

29	29.3	29.3	29.6	28.9	23.7	23.2	23.2	23.0
92	35.0	34.5	34.9	32.8	28.3	26.8	26.8	26.6
176	37.8	38.4	39.6	37.3	30.9	31.4	30.9	28.3
Body Weight (% of Control)								
176	100	101.6%	104.8%	98.7%	100%	101.6%	100%	91.6%

For heterozygous mice fed a standard diet (Groups 2, 6 and 7): The mean initial and final body weights of control (Group 2) males were 24.3 and 36.9 g, respectively. The mean initial and final body weights of control (Group 2) females were 19.0 and 28.7 g, respectively. There were no test article-related effects on mean body weight in females at 30 mg/kg/day. Treatment-related decreases in mean body weight were observed in males (5-8%) at 30 mg/kg/day and in females (5-8%) at 100→60 mg/kg/day. The following table shows the body weight data for Groups 2, 6 and 7.

Day	Males (Iron supplemented)			Females (Iron supplemented)		
	Group 2	Group 6	Group 7	Group 2	Group 6	Group 7
-3	24.3	24.5	19.0	19.0	18.7	19.0
15	27.7	27.7	22.3	22.9	22.7	22.3
29	29.1	28.6	22.4	23.9	23.2	22.4
92	33.2	30.8	26.0	26.4	24.3	23.2
176	36.9	34.5	26.4	28.7	27.6	23.4

For the wild type mouse supplemented with iron (Groups 1, and 3): The mean initial and final body weights of control (Group 1) males were 24.5 and 41.5 g, respectively. The mean initial and final body weights of control (Group 1) females were 20.0 and 36.5 g, respectively. There were no test article-related decreases in mean body weight in males at 200 mg/kg/day. In the females at 300 mg/kg/day, slight, statistically significant test article-related decreases (up to 12%) in mean body weight were observed starting on Day 29 and persisted through Day 176.

Day	Males (Iron supplemented)		Females (Iron supplemented)	
	Group 1	Group 3	Group 1	Group 3
-3	24.5	24.6	20.0	19.9
15	29.0	29.6	24.8	24.3
29	30.3	30.9	26.3	29.2
92	36.8	36.2	29.3	29.3
176	41.5	40.2	36.5	32.7

For the *p*-cresidine mice: The *p*-cresidine mice exhibited decreases in mean body weight in both sexes at 400 mg/kg/day throughout the treatment period (decreases of 4- 28% in the males and 4-18% in the females).

Food Consumption:

For heterozygous mice supplemented with iron (Groups 1, 3, 4 and 5): The mean initial and final food consumption in control (Group 1) males were 6.9 and 4.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 8.5 and 5.4 g/animal/day, respectively. There were no significant treatment-related effects.

For heterozygous mice fed a standard diet: The mean initial and final food consumption in control (Group 2) males were 5.6 and 3.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 6.2 and 4.0 g/animal/day, respectively. There were no significant treatment-related effects.

For the wild type mouse mice supplemented with iron: The mean initial and final food consumption in control (Group 1) males were 7.8 and 5.7 g/animal/day, respectively. The mean initial and final food consumption in control females were 8.2 and 6.7 g/animal/day, respectively. There were no test article-related effects.

For the *p*-cresidine mice: Mice treated with *p*-cresidine displayed decreases in food consumption throughout the treatment period.

Ophthalmology: Ophthalmoscopic examinations performed during Week 4 of the study revealed no treatment-related changes. However, ophthalmoscopic examinations performed during Week 15 of the study revealed treatment-related bilateral cataracts in Group 5 (feed supplemented with iron, 200 mg/kg/day for males and 300 mg for females) mice [two of 25 males at 200 mg/kg/day (animal nos. 5020 and 5021 and 2 of 22 females at 300 mg/kg/day (animal nos. 5501 and 5518)]. Ophthalmoscopically, these cataracts were characterized as total opacification of the lens fibers (mature cataracts).

At Week 26, several other mice from Group 5 were similarly affected (4 of 25 males, animal nos. 5001, 5007, 5020 and 5021 and 4 of 17 females, animal nos. 5508, 5511, 5512 and 5514). The lenticular change was identical to those seen earlier (bilateral mature cataracts). At this time, one female (1 of 17, animal no. 5513) and two males (2 of 25, animal nos. 5014 and 5022) of the heterozygous Group 5 mice had incipient/early cataracts. All affected mice (with the exception of the mice affected with incipient/early cataracts) had a visual deficit (blindness). In addition, at Week 26, one wild type female mouse (animal no. 3507 at 300 mg/kg/day) was also observed with bilateral mature cataracts.

Palpable Mass: There was no apparent increase in the incidence of palpable masses observed during the course of the study or prior to scheduled necropsy. A summary of

the palpable mass incidence at the last scheduled necropsy is presented in the following table (page 37 and 38 of the study report).

Table 4-5 Palpable mass incidence in heterozygous mice

Group Dose (mg/kg/day)	Males	Females
1 (0) with iron	0/25	1/24
2 0 (without iron)	0/25	0/25
3 20 / 30 (with iron)	0/23	0/24
4 100 (with iron)	0/23	0/23
5 200 / 300 (with iron)	0/25	0/17
6 30 / 100→60 (without iron)	1/24	0/19
7 400 p-cresidine (without iron)	0/23	0/25
8 0 (without iron)	NA	0/25
9 30 (without iron)	NA	0/24

Table 4-6 Palpable mass incidence in wild type mice

Group Dose (mg/kg/day)	Males	Females
1 0 (with iron)	0/25	0/25
2 0 (without iron)	0/25	0/25
3 200 / 300 (with iron)	0/24	0/24

Hematology: No significant treatment-related changes were observed.

Serum Iron Analysis: Test article-related serum chemistry alterations in the iron-supplemented p53 heterozygous mice included increased iron in both sexes (118-176% of control in males, 127-189% of control, control male = 128 µg/dl; control female = 129 µg/dl) at doses ≥100 mg/kg/day, increased total iron binding capacity (TIBC) in males (104-118% of control, control 313 µg/dl) at doses 200 mg/kg/day and in females (103-

141% of control, control = 343 $\mu\text{g}/\text{dl}$) at 300 mg/kg/day, and decreased unsaturated iron binding capacity (UIBC) in males (73-95% of control, control = 187 $\mu\text{g}/\text{dl}$) at 200 mg/kg/day. In the heterozygous mice fed a standard diet treatment-related effects were limited to increased iron (iron: 182-187% of control, control = 104-105 $\mu\text{g}/\text{dl}$) and decreased UIBC concentrations (63-84% of control, control = 231-242 $\mu\text{g}/\text{dl}$) in both sexes at 30 mg/kg/day (iron: 187% of control, control = 104 $\mu\text{g}/\text{dl}$) in males and in females at 100→60 mg/kg/day. In the iron supplemented wild type mice, effects were also limited to increased iron (148-159% of control, control = 137 $\mu\text{g}/\text{dl}$ for males and 154 $\mu\text{g}/\text{dl}$ for females) and increased TIBC (116-119% of control, control = 342 $\mu\text{g}/\text{dl}$) concentrations in males at 200 mg/kg/day and females at 300 mg/kg/day.

Tissue Iron Analysis: Iron supplementation of the p53 heterozygous control mice produced increases (approximately 30-50%) in liver and kidney iron content when compared to the control mice fed the standard rodent diet. Iron supplementation of the wild type control mice also produced increases in mean liver iron content (approximately 40- 50%) and mean kidney iron content (approximately 30%) in males.

In the iron supplemented p53 heterozygous mice, test article-related decreases in mean liver iron content (approximately 74-81%) and kidney iron content (approximately 40-55%) were noted in both sexes at doses ≥ 100 mg/kg/day when compared to iron supplemented controls as well as unsupplemented controls (decreases of 50-70% in the liver and 20-30% in the kidney). At doses of 20 mg/kg/day (males) and 30 mg/kg/day (females), decreases in mean liver and kidney iron content were only observed when compared to the iron supplemented controls (liver content was decreased by approximately 20- 30% and mean kidney iron content was decreased by approximately 30% for males and 10% for females).

In the p53 heterozygous mice receiving standard/unsupplemented diet, treatment-related decreases (approximately 51-64%) in mean liver iron content were observed in males at 30 mg/kg/day and in females at 100→60 and 30 mg/kg/day. Mean kidney iron content was also decreased by about 32% in the males at 30 mg/kg/day and by about 24-33% in females at doses of 100→60 and 30 mg/kg/day.

In the wild type mice, receiving iron-supplemented diet, treatment-related decreases in mean liver iron content were observed in both sexes (by approximately 71-77% when compared to the iron supplemented controls and by approximately 50% when compared to the unsupplemented controls). Mean kidney iron content also appeared to be decreased by about 60% in both sexes when compared to iron-supplemented controls and by about 40-57% when compared to the unsupplemented controls.

Organ Weights: Treatment-related organ weight changes were observed in the kidney and thymus. Kidney weights were increased in iron-supplemented p53 heterozygous and wild type males and females treated at 200 (107% of control, control = 0.430 g) and 300 mg/kg/day (108% of control, control = 108 g), respectively, and in non-supplemented p53 heterozygous females treated at 100→60 mg/kg/day (109% of control) when compared to control (0.308 g). Thymus weights were decreased in iron-supplemented,

p53 heterozygous males and females treated at 200 (86% of control, control = 0.029 g) and 300 mg/kg/day (72% of control, control = 0.043 g), respectively, when compared to control.

Macroscopic Examinations: Overall treatment-related macroscopic changes included eye opacity, pale discoloration of the kidneys, and dark foci in the stomach. In addition, *p*-cresidine-treated p53 heterozygous male mice had an increased incidence of thickening of the bladder and both sexes had a variety of macroscopic renal abnormalities, including discoloration, cystic change and pitted surface.

Histopathology:

Non-Neoplastic: Treatment-related non-neoplastic findings were observed in the eye (cataract at 200 and 300 mg/kg/day in iron-supplemented p53 heterozygous males and females, respectively; at 100→60 mg/kg/day in unsupplemented heterozygous female mice, and at 300 mg/kg/day in wild type females), kidney (diffuse tubular vacuolation, loss of normal focal tubular vacuolation, increased basophilic tubules), liver (biliary hyperplasia/periportal inflammation), stomach (increased severity of erosion in the glandular stomach and hyperkeratosis and ulcer in the non-glandular stomach), and skeletal muscle (degeneration/regeneration). The following table (from page 40 of the study report) shows the selected treatment-related histopathology findings in different groups.

Table 4-7 Selected test article-related findings

	p53 heterozygous + iron		p53 heterozygous no iron		Wild type + iron	
	Dose*		Dose*		Dose*	
	M	F	M	F	M	F
Eye: Cataract	200	300	-	100→60	-	300
Kidney:						
Diffuse tubular vacuolation	-	300	30	100	200	-
Loss of normal focal tubular vacuolation	≥100	-	30	-	200	-
Increased basophilic tubules	-	-	30	100→60	-	-
Liver:						
Biliary hyperplasia/periportal inflammation	-	-	-	100	-	-
Skeletal muscle:						
Degeneration/regeneration	200	300	-	-	200	300
Stomach:						
Glandular stomach: ↑ severity erosion	-	300	-	-	-	-
Non-glandular stomach: Hyperkeratosis	-	≥100	-	-	-	300
Non-glandular stomach: Ulceration	-	-	-	100	200	-

* Dose (mg/kg/day) at which finding or changed incidence of finding was observed

Best Possible Copy

The following table shows the incidences of histopathological observations in heterozygous mice with and without iron-supplementation including *p*-cresidine control.

Tissue Findings	MALE							FEMALE						
	GROUPS*							GROUPS						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
EYE														
Atrophy, retina, minimal					1									
Cataract, minimal					2							1		
Cataract, slight					1							4	1	
Cataract, moderate					4							8		
Cataract, marked					1									
Mineralization, lens, slight					1									
Mineralization, cornea	1		1	3										
Necrosis, single cell, lens, minimal							1					1		
KIDNEY														
Loss of normal vacuolation, tubular, epithelium, focal	23	21	16	3			2					1	2	
Vacuolation, tubular, epithelium, diffuse						1						1	2	
Basophilic tubules	10	7	8	8	15	15	14							
LIVER														
Biliary hyperplasia											1		6	2
SKELETAL MUSCLE														
Degeneration, myofiber, focal			2		21									
STOMACH														
Erosion, glandular	7	5	4	5	8	8		6	6	7	6	8	8	8
Hyperkeratosis			2					1	2	4	6	14		1

*: Groups:

Group 1: Control, Iron-supplemented feed

Group 2: Control, Standard feed

Group 3: Male: 20 mg/kg/day, Female: 30 mg/kg/day (Iron-supplemented feed)

Group 4: Male: 100 mg/kg/day, Female: 100 mg/kg/day (Iron-supplemented feed)

Group 5: Male: 200 mg/kg/day, Female: 300 mg/kg/day (Iron-supplemented feed)

Group 6: Male: 30 mg/kg/day, Female: 100→60 mg/kg/day (Standard feed)

Group 7: p-Cresidine 400 mg/kg/day (Standard feed)

The following table shows incidences of histopathological findings in wild type mice.

Tissue Findings	MALE						FEMALE					
	Dose (mg/kg/day)						Dose (mg/kg/day)					
	0	0	200				0	0	300			
EYE												
Cataract, minimal									1			
Cataract, slight									1			
Cataract, moderate									1			
Cataract, marked									1			
KIDNEY												
Loss of normal vacuolation, tubular, epithelium, focal	20	22						1	2			
Vacuolation, tubular, epithelium, diffuse			1									
Basophilic tubules	7	11	9				6	5	8			
SKELETAL MUSCLE												
Degeneration, myofiber, focal			4						2			
Regeneration, myofiber, focal	1	1	10				1	1	3			
STOMACH												
Erosion, glandular	5	2	3				3	3	6			
Hyperkeratosis							3	1	14			
Ulceration, nonglandular			1						1			

Neoplastic: There were no treatment-related neoplastic findings. The tumor incidences are shown in the following table. It is to be mentioned here that the sponsor provided limited historical control data for p53 heterozygous and wild type mice from three studies conducted at this facility during the period of 1999-2003. The sponsor did not perform statistical analysis of the tumor incidences.

MALE							
Organ/Tumor	IRON-SUPPLEMENTED FEED				NON-SUPPLEMENTED FEED		
	0 mg/kg/day (n = 25)	20 mg/kg/day (n = 25)	100 mg/kg/day (n = 25)	200 mg/kg/day (n = 25)	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)	P-Cresidine (400 mg/kg/day) (n = 25)
GROUP	1	3	4	5	2	6	7
Heart							
Lymphoma		1 (4%)					

Stomach							
Lymphoma		1 (4%)					
Duodenum							
Lymphoma		1 (4%)					
Ileum							
Lymphoma		1 (4%)					
Cecum							
Lymphoma							
Pancreas							
Lymphoma		1 (4%)					
Liver							
Lymphoma		2 (8%)					
Transitional cell carcinoma							1 (4%)
Adrenal							
Lymphoma		1 (4%)					
Pheochromocytoma							1 (4%)
Cortical adenoma			1 (4%)		1 (4%)	1 (4%)	
Pituitary							
Lymphoma		2 (4%)					
Epididymides							
Lymphoma		1 (4%)					
Leiomyosarcoma						1 (4%)	
Kidney							
Lymphoma		2 (8%)					
Prostate							
Transitional cell carcinoma							2 (8%)
Urinary Bladder							
Metaplasia, squamous, epithelium, focal, minimal							10 (40%)
Same, slight							6 (24%)
Same, moderate							5 (20%)
Squamous cell carcinoma							1
Transitional cell carcinoma							16 (64%)
Transitional cell papilloma							10 (40%)
Bone Marrow							
Lymphoma		2 (8%)					
Lymph Node-Axillary							
Lymphoma		2 (8%)					
Lymph Node-Bronchial							
Lymphoma		2 (8%)					
Lymph Node-Mandibular							
Lymphoma		2 (8%)					
Lymph Node- Mesenteric							
Lymphoma		2 (8%)					

Thymus							
Lymphoma		2 (8%)				1 (4%)	
Spleen							
Lymphoma		2 (8%)					
Systemic/Any Site							
Lymphoma		2 (8%)				1 (4%) [3.8%]*	
Preputial gland							
Metaplasia, squamous, focal, minimal							1 (4%)
Skin							
Carcinoma							1 (4%)
Bone-Sternum							
Lymphoma		1 (4%)					
Brain							
Lymphoma		1 (4%)					
Lung							
Lymphoma		2 (8%)					
Transitional cell carcinoma							1 (4%)
Trachea							
Lymphoma		1 (4%)					
Eye							
Lymphoma		1 (4%)					
Harderian Gland							
Adenoma							1 (4%)

*: Historical control data from this testing facility from 1999-2003

FEMALE							
Organ/Tissue Finding	IRON-SUPPLEMENTED				NON-SUPPLEMENTED		
	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)	100 mg/kg/day (n = 25)	300 mg/kg/day (n = 25)	0 mg/kg/day (n = 25)	100→60 mg/kg/day (n = 25)	p-Cresidine (400 mg/kg/day) (n = 25)
GROUP	1	3	4	5	2	6	7
Heart							
Lymphoma		1 (4%)					
Stomach							
Lymphoma						1 (4%)	
Pancreas							
Acinar cell adenocarcinoma						1 (4%)	
Liver							

Lymphoma		1 (4%)					
Hepatocellular adenoma							1 (4%)
Pituitary							
Lymphoma		1 (4%)					
Kidney							
Lymphoma		1 (4%)					
Ovary							
Lymphoma		1 (4%)					
Urinary Bladder							
Metaplasia, squamous, epithelium, focal, minimal							7 (28%)
Same, slight							14 (56%)
Transitional cell carcinoma							5 (20%)
Transitional cell papilloma							6 (24%)
Papilloma, squamous cell							2 (8%)
Uterus							
Osteosarcoma					1 (4%)		
Bone Marrow							
Lymphoma		1 (4%)					
Lymph Node							
Lymphoma, bronchial		1 (4%)				1 (4%)	
Lymphoma, pancreatic						1 (4%)	
Thymus							
Lymphoma	1 (4%)	1 (4%)					2 (8%)
Spleen							
Lymphoma		1 (4%)					1 (4%)
Systemic/Any Site							
Lymphoma	1 (4%)	1 (4%)					2 (8%) [5.4%]*
Mammary Gland							
Adenocarcinoma, onset day = 121			1 (4%)				
Skin							
Osteosarcoma, onset day = 172	1 (4%)						
Lung							
Lymphoma	1 (4%)	1 (4%)					1 (4%)

*: Historical control data from this testing facility from 1999-2003

The following table shows the neoplastic findings in Groups 8 and 9 (Standard Feed, Females)

Organ/Tissue	FEMALES (Non-Supplemented Feed)	
	0 mg/kg/day (n = 25)	30 mg/kg/day (n = 25)
Digestive System		
Lymphoma, salivary gland	1 (4%)	
Hemolymphatic System		
Lymphoma, lymph node, mesenteric	1 (4%)	

The following table shows the neoplastic findings in the wild type animals.

ORGAN/TISSUE FINDINGS	MALES (n = 25)			FEMALES (n = 25)		
	+ Fe	-Fe	+Fe	+ Fe	-Fe	+Fe
Dose (mg/kg/day)	0	0	200	0	0	300
Hepatobiliary System						
Histiocytic sarcoma				1 (4%)		
Genitourinary System						
Endometrial stromal polyp				1 (4%)	1 (4%)	
Hemolymphatic System						
Histiocytic sarcoma				1 (4%)		
Lymphoma, lymph node, axillary				1 (4%)		
Lymphoma, lymph node, bronchial				1 (4%)		
Histiocytic sarcoma, lymph node, bronchial				1 (4%)		
Lymphoma, lymph node, mandibular				1 (4%)		
Lymphoma, lymph node, mediastinal				1 (4%)		
Lymphoma, lymph node, mesenteric				1 (4%)		
Lymphoma, lymph node, renal				1 (4%)		
Lymphoma, lymph node, sublumbar				1 (4%)		
Histiocytic sarcoma, thymus				1 (4%)		
Histiocytic sarcoma, spleen				1 (4%)		
Systemic/Any Site						
Mast cell tumor			1 (4%)			

Lymphoma				1 (4%) [1.8%]*		
Histiocytic sarcoma				1 (4%)		
Respiratory system						
Histiocytic sarcoma, lung				1		

*: Historical control data from this facility from 1999-2003

Toxicokinetics: Based on AUC_(0.5-6h)/dose values, there appeared to be no significant gender differences in exposure of p53 heterozygous type mice supplemented with dietary iron. However, in non-supplemented animals, there was an apparent gender difference in drug exposure (females were more exposed than males). At the low dose level, the exposure of p53 heterozygous mice was lower in males and higher in the females when food was not supplemented with iron. Exposure appeared to be over proportional between doses of 20 to 200 mg/kg/day in p53 heterozygous males and between doses of 30 to 300 mg/kg/day in p53 heterozygous females, when feed was supplemented with iron. Female p53 wild type mice were less exposed to ICL670 at the high dose when compared to heterozygous mice. Mean TK parameters are shown in the following table (from page 43 of the study report).

Table 4-8 Mean toxicokinetic parameters of total ICL670 in mouse

	Dose (mg/kg/day)		C _{max} /Dose (µg/mL)/(mg/kg/day)		AUC _(0.5-6h) /Dose (µg*hours/mL)/(mg/kg/day)	
	Male	Female	Male	Female	Male	Female
Iron supplemented feed						
Group 3 (Low dose group)	20	30	0.655	0.590	1.98	1.90
Group 4 (Mid dose group)	100	100	0.620	0.859	2.03	2.07
Group 5 (High dose group)	200	300	0.910	1.15	3.19	3.66
Group 3w* (High dose group)	200	300	*	0.500	*	1.93
Iron non-supplemented feed						
Group 6 (Low dose group)	30	100→60	0.560	0.907	1.30	3.24
Group 9 (Low dose group)	-	30	-	0.883	-	3.29

*Group 3w (wild type mice). Due to the lack of data, no toxicokinetic analysis was performed for males in this group

Summary Study Findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The dose selection was in accordance with the ExecCAC recommendations (Appendix-3, 4, and 5). The positive control, *p*-cresidine, produced expected tumor responses in the urinary bladder. It is to be mentioned here that the sponsor provided limited historical control data for p53 heterozygous and wild type mice

from three studies conducted at this facility during the period of 1999-2003. The sponsor did not conduct statistical analysis of the tumor data.

ICL670 was found to be negative in the Ames assay and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes, positive in micronucleus assay in V79 cells (non-GLP). In the *in vivo* rat (non-supplemented) bone marrow micronucleus test by oral route, ICL 670 was found to be positive at 500 mg/kg dose level. In another oral bone marrow micronucleus test in iron-overloaded rats, ICL670 was shown to be negative at 500 mg/kg dose. At 300 mg/kg dose, ICL 670 was also shown to be negative in the rat bone marrow micronucleus test irrespective of the iron-overloading of the rats. The appropriateness of the 26-week p53 (+/-) transgenic mouse assay was discussed at the CDER PTCC on August 16, 2001. The Committee considered that the positive genotoxicity results of the micronucleus assay in non-iron-supplemented rats are relevant and recommended that it is appropriate to conduct p53 (+/-) transgenic mouse assay in both iron supplemented and non-iron-supplemented animals, which will help in the interpretation of study results. Overall, the study was designed according to ExecCAC recommendations and appeared to be adequate and acceptable.

Evaluation of tumor findings: There were no test article-related neoplastic findings. The sponsor did not conduct statistical analysis of the tumor data. Based on the results from this study, ICL670 administered orally via gavage for 26 weeks was not carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and at doses up to 300 mg/kg/day for females irrespective of iron supplementation. As expected, the administration of *p*-cresidine caused proliferative changes notably in the urinary bladder of p53 heterozygous mice.

Carcinogenicity Summary: ICL670 was administered orally via gavage as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received *p*-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The doses for the current study were selected based on MTD and as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 17, 2002 and November 12, 2002, Appendix-3, 4, and 5, respectively).

Test article-related moribundity/mortality occurred in one high dose p53 heterozygous female at 300 mg/kg/day with dietary iron supplementation and one high dose p53 heterozygous female at 100 mg/kg/day without dietary iron supplementation. Nine additional p53 heterozygous mice (four females, supplemented with dietary iron at 300 mg/kg/day and five females, without dietary iron supplementation at 100 mg/kg/day) were also considered test article-related. Test article-related moribundity was also observed in one high-dose wild type male at 200 mg/kg/day with dietary iron supplementation. Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female mouse at 300 mg/kg/day. There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation. The positive control, *p*-cresidine, produced effects, which included proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%). Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were confined generally to non-supplemented mice.

Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Carcinogenicity Conclusions: Based on the results from this study, ICL670 administered orally via gavage for 26 weeks does not appear to be carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females either in the presence or absence of iron supplementation.

Recommendations for further analysis: None.

Labeling Recommendations: None

Addendum/Appendix Listing:

Appendix-3: Executive CAC meeting minutes dated August 21, 2001, page: 316

Appendix-4: Executive CAC meeting minutes dated September 17, 2002, page: 321

Appendix-5: Executive CAC meeting minutes dated November 12, 2002, page: 326

Appendix-6: List of neoplastic and non-neoplastic lesions, page: 332

Dose-Ranging Study Report: None

CAC Report: None

Alternative Study Protocols and CAC Report: None

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2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

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Reproductive Toxicology**Rat****Segment I Oral Fertility and Early Embryonic Development Study in Rats (Study No. 987102)**

Testing Laboratory: Novartis Pharmaceutical Corporation
East Hanover, NJ, USA.

Date Started: October 12, 1998

Date Completed: December 21, 1998

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Wistar Hanover rats were used in this study. Male rats were 11 weeks old and had a body weight range 294-361 g. Female rats were 10 weeks old and had a body weight range of 171-222 g.

Drug Batch: ICL 670, Lot No. 97902.

Methods: In a segment I fertility and reproductive performance study, male and female rats (25/sex) were administered with ICL 670 by oral gavage at daily doses of 7.5, 25 and 75 mg/kg/day (dose volume was 10 ml/kg). The control group received 0.5% aqueous Klucel suspension. The dose selection was based on the results of a 4-week oral toxicity study in rats (study no. 974080). In that study, rats were treated with ICL 670 at daily doses of 10, 30, and 100 mg/kg and a total of 8 treatment-related death were observed in the 100 mg/kg group. Based on the results of this study, a high dose of 75 mg/kg was expected to produce signs of paternal toxicity and 7.5 mg/kg was expected to be the NOEL. A 4-week pre-mating treatment period was selected for the males, as there was no testicular histopathology findings in the 4-week toxicity study. Males were treated once daily for at least 28 days prior to mating (1:1), during the 2-week mating period, and until terminal sacrifice (between Day 50 and Day 54). Females were treated once daily for 14 days prior to mating, during the 2-week mating period and through gestation day 6. All animals were observed daily for mortality and clinical signs. Food consumption and body weights were recorded on treatment days 1, 4, 8, 11, 15, 18, 22, 25 and 29 in males and on pre-mating treatment days 1, 4, 8, 11 and 15 and gestation days 0, 3, 6, 9 and 13 in females. During the mating period, vaginal smears were collected from females daily for determination of estrus stage until detection of sperm. On day 13 of gestation, females were sacrificed and subjected to macroscopic examinations. The number of corpora lutea, number and distribution of implantation sites, number of live and dead fetuses and number of early and late resorptions were determined. Male rats were sacrificed between study days 50 and 54. After weighing testis and epididymis, the left testis was frozen until determination of homogenization-resistant spermatids (sperm head counts). The number of dead and motile sperms was evaluated.

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Results:

1. **Clinical Signs:** The only treatment-related clinical signs observed in this study was decreased stool in males at 75 mg/kg dose.
2. **Mortality:** One male rat died at 75 mg/kg dose on study day 17.
3. **Body Weight and Food Consumption:** Treatment-related decrease in mean body weight values were observed from day 4 (96% of control; control = 341 g) to day 53 (87% of control; control = 407 g) in males at 75 mg/kg dose. In females, body weights were not significantly changed due to treatment. Food consumption was significantly decreased from treatment day 4 (90% of control; control = 107 g) to treatment day 18 (87% of control; control = 102 g) in males at 75 mg/kg dose level. No treatment-related change in the food consumption was noted in females.
4. **Organ Weights:** None.
5. **Gross Pathology:** There were no treatment-related necropsy findings in this study in either sex.
6. **Mating, Fertility, and Reproductive Parameters:** There were no treatment-related effects on estrous cycles, precoital intervals, pregnancy rate or mating indices at any dose level. There were no effects of treatment on percent motile sperm or sperm count at any dose level. There was no effect of treatment on any of the examined reproductive parameters in this study. Fertility and reproductive parameters for rats that received ICL 670 by oral gavage at doses of 7.5, 25, and 75 mg/kg/day are shown in the following table:

Parameter	0 mg/kg/day	7.5 mg/kg/day	25 mg/kg/day	75 mg/kg/day
N=	25	25	25	25
Mating Index, %	96 (24/25)	100 (25/25)	100 (25/25)	96 (24/25)
Pregnant Dams	23	22	24	23
Fertility Index, %	92 (23/25)	88 (22/25)	96 (24/25)	92 (23/25)
Corpora lutea/dam	14.5 (319/22)	13.6 (299/22)	13.4 (321/24)	13.7 (315/23)
Implant sites/dam	13 (285/22)	12.4 (272/22)	12.1 (290/24)	12.4 (286/23)
Pre-implantation loss, %	10.7	9.0	9.7	9.2
Post-implantation loss, %	6.7	8.1	7.9	4.5
Resorptions				
- Total	0.9	1.0	1.0	0.6
- early	0.9	1.0	1.0	0.6
- late	0.0	0.0	0.0	0.0
Live fetuses/dam	12.1	11.4	11.1	11.9
Dead fetuses/dam	0	0	0	0
Total spermatids (x10 ⁶)/g testis	96.05	97.452	103.645	100.96
Sperm motility, %	84.6	87.4	87.8	83.3

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In a segment I fertility and reproductive performance study in rats, male and female rats received ICL 670 by oral gavage at doses of 7.5, 25, and 75 mg/kg/day. Male rats were treated with ICL 670 for 28 days prior to mating, during the mating period and until terminal sacrifice (between study days 50 and 54). Female rats received ICL 670 for 14 days prior to mating, during the 2-week mating period and through gestation day 6. There were no adverse effects on mating, fertility or reproductive parameters including early embryonic development at any dose level in rats.

Embryofetal development

Segment II Oral Embryo-Fetal Development Study in Rats (Study No. 987055)

Testing Laboratory: Novartis Pharmaceuticals Corporation
East Hanover, NJ, USA.

Date Started: June 05, 1998

Date Completed: July 02, 1998

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Wistar rats were used throughout the study. Pregnant females were approximately 10-11 weeks old at the start of dosing and had a body weight range of 183-255 g.

Methods: In a Segment II reproductive toxicity study, pregnant female rats (25/group) received ICL 670 by oral gavage at 10, 30, and 100 mg/kg doses once daily on gestation day 6 through 17. The dose volume was 10 ml/kg. The dose selection was based on the results of the 4-week oral (gavage) toxicity study in rats (study no. 974080). In that study, rats were treated with ICL 670 at daily doses of 10, 30, and 100 mg/kg and a total of 8 treatment-related death were observed in the 100 mg/kg group. Based on the results of this study, a high dose of 100 mg/kg was expected to produce signs of maternal toxicity and 10 mg/kg was expected to be the NOEL, and the mid-dose of 30 mg/kg was expected to show intermediate level of toxicity. The control group receive 0.5% Klucel at an equivalent dose volume (10 ml/kg). All animals were observed daily for mortality and clinical signs. Food consumption and body weights were recorded on gestation days 3, 6, 9, 12, 15, 18, 21. On gestation day 21, females were sacrificed and subjected to macroscopic examinations. The number of corpora lutea, number and distribution of implantation sites, number of live and dead fetuses and number of early and late resorptions were determined. Approximately one half of the fetuses from each litter were placed in fixative for visceral examination and the remaining fetuses were placed in 70% ethanol and subsequently stained with alizarin red for skeletal examinations.

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Results:

1. **Clinical Signs:** Treatment-related clinical signs were observed at 100 mg/kg dose that included piloerection, ptosis, and bifateral red tears and decreased or no stool.
2. **Mortality:** Two animals at 100 mg/kg died on gestation day 17 and 18.
3. **Body Weight and Food Consumption:** Body weights were reduced at 100 mg/kg on gestation day 6 (97% of control; control = 221 g) and 21 (91.5% of control; control = 330 g). Food consumption was also decreased at 100 mg/kg dose on gestation day 6 (83% of control; control = 69 g/animal) to 18 (73% of control; control = 78 g/animal). There was a statistically significant reduction in food consumption at 30 mg/kg dose level on gestation days 6-9 (91% of control; control = 69 g/animal).
4. **Gross Pathology:** One of the dead animals at 100 mg/kg had a distended stomach and orange-colored intestines filled with gas/fluid, and another dead rat had a distended stomach and vagina was filled with mucoid yellow exudate. Coagulated blood in the uterus was noted in one female each at 0, 30, and 100 mg/kg.
5. **Reproductive Parameters:** There were no treatment-related changes in the reproductive parameters including the mean number of viable fetuses, early and late resorptions, post-implantation loss and fetal sex ratios. Reproductive parameters for rats that received ICL 670 by oral gavage at doses of 10, 30, and 100 mg/kg are shown in the following table:

Parameter	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of pregnant dams	21	24	21	23
Corpora lutea/dam	13.1 (263/20)	12.8 (295/23)	13.2 (278/21)	13.1 (263/20)
Pre-implantation loss, %	10.6	15.6	14.0	14.8
Implantation sites/dam	11.8 (235/20)	10.8 (249/23)	11.4 (239/21)	11.2 (224/20)
Post-implantation, %	6.8	7.6	7.9	7.6
Embryonic resorptions/dam				
- Total	0.8	0.8	0.9	0.9
- Early	0.8	0.8	0.9	0.8
- late	0.1	0.0	0.0	0.1
Live fetuses/dam	10.9 (219/20)	10.0 (230/23)	10.5 (220/21)	10.4 (207/20)
Dead fetuses	0	0	0	0
Viable Male Fetuses, %	46	52	51	52
Male fetal body weight (g)	5.1	5.1	5.2	4.8
Female fetal body weight (g)	4.8	4.8	4.9	4.6

6. **External, Visceral, and Skeletal Examinations of Fetuses:** The summary of fetal external, visceral, and skeletal malformations and variations are shown in the following table:

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Parameter	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Fetuses/Litters Examined	219/20	230/23	220/21	207/20
Fetal External Malformation				
Proboscis	0	0	0	1
Exencephaly	0	0	0	1
Protruding tongue	0	0	0	1
Maxillary micrognathia	0	0	0	1
Omphalocele	1	0	0	1
Fetal Visceral Malformations				
Fetuses/Litter Examined	104/20	109/23	104/21	96/20
Eye (s) malpositioned	0	0	0	1
Fetal Visceral Variations				
Renal Papilla-small	3	2	0	4
Ureter-dilated	1	0	0	2
Ureter-convoluted	0	0	1	0
Fetal Skeletal Malformations				
Fetuses/Litter Examined	115/20	121/23	116/21	110/20
Maxilla-fused	1	0	0	0
Zygomatic-absent	1	0	0	0
Zygomatic-fused	1	0	0	0
Rib-wavy	6	12	2	4
Fetal Skeletal Variations				
Exoccipital-incomplete ossification	3	1	5	4
Frontal-incomplete ossification	1	2	1	1
Hyoid-incomplete ossification	2	1	1	2
Hyoid-unossified	0	1	0	0
Interparietal-incomplete ossification	12	12	12	9
Maxilla-incomplete ossification	2	3	0	0
Nasal-incomplete ossification	1	0	0	2
Palatine-incomplete ossification	1	0	0	0
Parietal-incomplete ossification	8	17	9	7
Squamosal-incomplete ossification	5	5	2	3
Supraoccipital-incomplete ossification	1	3	2	5

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Zygomatic-incomplete ossification	4	5	7	3
Metacarpal-incomplete ossification	2	0	0	1
Metacarpal-unossified	2	0	0	1
Forepaw phalanx-incomplete ossification	35	52	31	33
Forepaw phalanx-unossified	11	3*	3*	5
Sternebra-incomplete ossification	21	27	25	23
Sternebra- misshapen	5	10	9	10
Sternebra-unossified	2	4	0	2
Sternebra-extra ossification site	0	0	0	1
Sternebra-bipartite ossification	1	0	0	1
Rib-Supernumerary, short	37	34	32	33
Rib-Supernumerary, full	1	6	2	11*
Rib-thickened	3	8	2	4
Rib-cervical	1	1	0	1
Rib-incomplete ossification	1	0	0	0
Thoracic centrum-dumbell ossification	1	4	1	3
Thoracic Centrum-incomplete ossification	0	0	0	1
Lumbar Centrum-supernumerary	0	1	0	7**
Lumbar Centrum- bipartite ossification	0	0	0	1
Lumbar centrum-dumbell ossification	0	0	0	1
Lumbar vertebra-supernumerary	0	1	0	7**
Lumbar vertebra-incomplete ossification	1	0	0	0
Sacral vertebra- incomplete ossification	0	0	0	3
Metatarsal-unossified	0	0	0	1
Hindpaw phalanx-unossified	33	21*	19*	28
Hindpaw phalanx	43	36	36	39

* = <0.05
** = < 0.01

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7. **Toxicokinetics:** In this study, ICL 670 was administered to pregnant rats (5/dose) at dose levels of 10, 30 and 100 mg/kg once daily by oral gavage from gestation days 6 through 17. Blood samples were taken on gestation day 17 for toxicokinetic analyses. The mean (n = 5) toxicokinetic parameters of ICL 670 in an oral embryo-fetal development study are summarized in the following table (from vol. 20, page 8-5201 of sponsor's submission):

Dose (mg/kg)	10	30	100
C _{max} (µg/ml)	8.59 γ 3.02	36.0 γ 5.8**	72.3 γ 14.0
T _{max} (hr)	1*	1*	3*
AUC (0-24h) (µg.h/ml)	24.1 γ 8.7	155 γ 39**	806 γ 92**

*: Median value.

**: n = 4.

Plasma concentrations were increased more than proportionately with the dose between 10 and 100 mg/kg based on the AUC values. At 100 mg/kg, the time to reach C_{max} was found to be higher at 100 mg/kg dose level.

In a Segment II reproductive toxicity study in rats, pregnant female rats received ICL 670 by oral gavage at doses of 10, 30, and 100 mg/kg/day from days 6 to 17 of gestation. Mortality was observed at 100 mg/kg dose with associated gastrointestinal lesions in two animals on gestation days 17 and 18. Clinical signs at 100 mg/kg/day included piloerection, decrease locomotor activity, decreased stool and ptosis. Decreased body weight and food consumption was observed at 100 mg/kg dose level. There were no treatment-related changes in reproductive parameters including the mean number of viable fetuses, early and late resorptions, post-implantation loss. Fetal body weights at 100 mg/kg in male (94% of control) and female (96% of control) were slightly low (not statistically significant) compared to control (male = 5.1 g; female = 4.8 g). This effect could be attributed to the reduced maternal body weights and food consumption at this dose level. In the 100 mg/kg group, one fetus each from three litters had external malformations: one fetus had orofacial malformations (proboscis, protruding tongue, and maxillary micrognathia); one fetus had encephaly and another fetus had omphalocele. One control fetus had omphalocele. These malformations were not considered to be treatment-related because of relatively low incidence, dissimilar in nature and they were not statistically (Fisher's Exact Test) significant from the concurrent control. There appeared to be no treatment-related visceral malformations in fetuses. There were no meaningful skeletal malformations among the groups. A significant number of skeletal variations were observed at 100 mg/kg/day (full supernumerary ribs, supernumerary lumbar vertebra and centrum). There is a paucity of historical control data in the Wistar Hannover rat; however, supernumerary rib has been shown to be a common skeletal variation in rodents (36-55% in Sprague-Dawley rats and 40% in C57BL mice). A significant number of skeletal variations were observed for treatment groups; however, there were no dose-response relationships and none of these effects should have any impact on survival and hence, no apparent indication of teratogenicity. The NOEL of ICL 670 for the rat maternal toxicity and the embryo-fetal development appeared to be 30 mg/kg/day.

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Rabbit**Segment II Oral Embryo-Fetal Development Study in Rabbits (Study No. 987056)**

Testing Laboratory: Novartis Pharmaceuticals Corporation
East Hanover, NJ, USA.

Date Started: June 17, 1998

Date Completed: July 17, 1998

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Pregnant Female White New Zealand rabbits were used in this study. At the start of dosing, animals were 23 weeks old with a body weight range of 2.96 to 4.42 kg.

Drug Batch: ICL 670, Batch number 97902.

Methods: In a Segment II teratogenicity study, pregnant female rabbits (20/group) received ICL 670 at daily doses of 5, 20 and 50 mg/kg by oral gavage on gestation days 7 through 20. The dose volume was 5 ml/kg. Control rabbits received 0.5% Klucel aqueous suspension. The dose selection was based on the results of a preliminary dose range-finding study (study no. 988036) in rabbits (25, 50, 100, and 200 mg/kg orally; 5 females/group; dosing period: gestation days 7-20). In this study, all five animals at 200 mg/kg died on gestation days 16-19. One female at 100 mg/kg was sacrificed moribund on gestation day 19 and two females aborted on gestation 19 and 25. Maternal toxicity was seen at 50 mg/kg including slight body weight loss and decrease food consumption and one female aborted at 50 mg/kg on gestation day 24. Macroscopic examinations revealed raised areas in gall bladder (2/5 animals at 100 mg/kg, 5/5 animals at 200 mg/kg) and thickening of the stomach (4/5 animals at 200 mg/kg and 1/5 animals at 100 mg/kg). One fetus at 50 mg/kg had omphalocele and there were no other external fetal malformations. Based on the results of this dose range-finding study, the high dose selected as 50 mg/kg and 5 mg/kg was expected to be the NOEL.

In the Segment II teratogenicity study in rabbits, clinical signs and mortality was observed daily. Food consumption was recorded daily on gestation days 5 through 29. Body weights were taken on gestation days 0, 5, 7, 10, 14, 17, 21, 24, and 29. Dams were sacrificed on gestation day 29. The ovaries and uterus were removed from each animal. Reproductive data were recorded as follows: number of dead fetuses, number of resorptions, number of live fetuses, number of corpora lutea in the ovaries, fetal body weight. Fetuses were examined externally for malformations and variations. Fetuses were stained with alizarin red and examined for skeletal anomalies.

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Results:

1. **Clinical Signs:** Treatment-related clinical signs were observed at 50 mg/kg dose, which included decreased stool and soft stool. One 20 mg/kg female aborted on gestation day 21 and one 50 mg/kg aborted on gestation day 26.
2. **Mortality:** None.
3. **Body Weight and Food Consumption:** At 50 mg/kg, the body weight was reduced from gestation day 7 (98.7% of control; control = 3704 g) to 29 (97.2% of control; control = 4107 g). There was a significant reduction in body weight gain at 50 mg/kg dose level (50% of control weight gain; control weight gain = 229 g) during gestation day 7-21. Food consumption was significantly decreased (78% of control; control = 218 g/animal/day) at 50 mg/kg during gestation days 16-17.
4. **Gross Pathology:** No treatment-related findings.
5. **Reproductive Parameters:** The percent of total resorption was increased slightly in the treated group when compared to control. Reproductive parameters for rabbits that received ICL 670 by oral gavage at doses of 5, 20, and 50 mg/kg are shown in the following table:

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	50 mg/kg/day
Number of pregnant dams	20	20	19	17
Corpora lutea/dam	10.2 (204/20)	10.1 (201/20)	9.5 (181/20)	10.2 (174/18)
Pre-implantation loss, %	9.8	12.4	11.6	9.8
Implantation sites/dam	9.2 (184/20)	8.8 (176/20)	8.4 (160/20)	9.2 (157/20)
Post-implantation, %	2.2	4.5	4.4	3.8
Embryonic resorptions/dam				
- Total	0.2	0.4	0.4	0.4
- Early	0.1	0.3	0.3	0.1
- late	0.1	0.1	0.1	0.2
Live fetuses/dam	9.0 (180/20)	8.4 (168/20)	8.1 (153/19)	8.9 (151/17)
Dead fetuses	0	0	0	0
Viable Male Fetuses, %	53	51	50	53
Male fetal body weight (g)	44.3	44.5	45.6	43.5
Female fetal body weight (g)	42.6	44.5	44.9	43.4

6. **External, Visceral, and Skeletal Examinations of the Fetuses:** There were no treatment-related external or visceral malformations or variations. There were no meaningful skeletal malformations among the groups. At 20 mg/kg, three fetuses from different litters had multiple thoracic vertebra malformations (hemivertebra, malpositioned and fused vertebra, misaligned, fused and misshapen centrum as well as hemicentrum, etc.). However, there

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were no skeletal malformations at 5 or 50 mg/kg dose levels. There appears to be no dose response relationship for the skeletal malformations observed at 20 mg/kg dose level. Summary of fetal skeletal malformations and variations in rabbits are shown in the following table:

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	50 mg/kg/day
Litters Evaluated	20	20	19	17
Fetuses Evaluated	180	168	153	151
Fetal External Malformations				
Omphalocele	1	1	0	0
Fetal Visceral Malformations				
Ventricular Wall-Enlarged	0	1	0	0
Anorchia	0	1	0	0
Gall bladder-Absent	1	0	0	0
Fetal Visceral Variations				
Liver-supernumerary lobe	0	1	0	0
Coagulated blood around bladder	1	0	0	0
Fetal Skeletal Malformations				
Rib absent (Unilateral)	0	0	1	0
Rib branched	1	0	0	0
Rib fused	1	0	0	0
Thoracic centrum-hemicentric	0	0	2	0
Thoracic centrum-misaligned	0	0	2	0
Thoracic centrum-misshapen	0	0	1	0
Thoracic centrum- fused	0	0	1	0
Thoracic vertebra-malpositioned	0	0	1	0
Thoracic vertebra-fused	0	0	1	0
Thoracic hemivertebra	0	0	3	0
Fetal Skeletal Variations				
Interparietal-incomplete ossification	0	1	0	0
Interparietal-bipartite ossification	0	1	0	0
Sternebra-extra ossification site	1	1	6	1
Sternebra-misshapen	1	3	3	2
Rib-short and detached	0	0	1	0
Rib-supernumerary, discontinuous	0	0	1	0

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Cervical centrum-incomplete ossification	1	2	1	2
Cervical centrum-unossified	0	1	0	0
Thoracic centrum-dumbell ossification	0	3	0	0
Lumbar centrum-supernumerary	1	6	6	3
Lumbar vertebra-supernumerary	1	6	6	3
Metatarsal-unossified	0	1	0	0

7. **Toxicokinetics:** Toxicokinetic parameters were determined from an oral embryo-fetal development study in rabbits. ICL 670 was administered once daily to rabbits (5 animals/dose) by oral gavage from gestation days 7 through 20 at dose levels of 5, 20 and 50 mg/kg. Blood samples were collected on gestation day 20 before dosing and at 1, 3, 6 and 24 hr post dose. The mean toxicokinetic parameters of total ICL 670 in the pregnant rabbits are summarized in the following table (from vol. 15, page 8-3900 of sponsor's submission):

Dose (mg/kg)	5	20	50
C _{max} (µg/ml)	0.517 γ 0.157	2.87 γ 0.72	12.6 γ 3.8
T _{max} (h)	3*	3*	3*
AUC (0-24h) (µg.h/ml)	5.58 γ 1.36	25.3 γ 4.1	219 γ 92

*: Median value.

There was a proportionate increase in C_{max} values from 5 to 20 mg/kg and found to be overproportionate in between 20 and 50 mg/kg dose levels based on AUC values.

In a segment II teratogenicity study in rabbits, pregnant female rabbits received ICL 670 by oral gavage at 5, 20, and 50 mg/kg doses from gestation day 7 through 20. Maternal toxicity (soft, decreased or no stool, decrease food consumption, lower body weight and lower body weight gain) was observed at 50 mg/kg dose level. ICL 670 found to have no effect on the reproductive parameters. Multiple thoracic vertebra malformations (hemivertebra, malpositioned and fused vertebra, misaligned, fused and misshapen centrum as well as hemicentrum, etc.) were observed at 20 mg/kg; however, there were no skeletal malformations at 5 or 50 mg/kg. These skeletal malformations observed at 20 mg/kg appeared to be incidental because of the lack of a dose-response relationship and seems not related to treatment. There were no meaningful differences in skeletal variations observed among the groups. However, none of these variations would be expected to have an impact on survival. Based on the results of these results, the NOEL for maternal toxicity appeared to be 20 mg/kg and the NOEL for embryo-fetal toxicity was established as 50 mg/kg and ICL 670 was not considered to be teratogenic in rabbits under the conditions of the study.

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Prenatal and postnatal development

Study Title: An Oral Pre- and Postnatal Development Study in Rats (Segment III)

Key Study Findings:

In an oral (gavage) Segment III study in rats, pregnant females were administered with ICL670 at 0, 10, 30 and 90 mg/kg/day. Females were treated once daily on gestation day (GD) 6 through lactation day (LD) 20. The 90 mg/kg/day dose group was terminated on LD 1 to 3 due to excessive dam/litter losses. There was a statistically significant increase in the number of stillborn pups at 90 mg/kg/day group. There was a statistically significant increase in the number of stillborn pups at 90 mg/kg/day group. There appears to be no treatment-related adverse effects on behavior in F1 pups. There was an increased incidence of renal anomalies (short/ absent renal papilla, fluid- filled kidney) in F1 male pups sacrificed at the end of the post-weaning phase of the study at 30 mg/kg/day. However, these effects were not considered as teratogenic effects, as kidney toxicity was also observed in the neonatal rat toxicity study and were considered as nephrotoxicity associated with ICL670. Overall, the NOAEL for Fo maternal toxicity appears to be 30 mg/kg/day and the NOAEL for fetal to toxicity appears to be 10 mg/kg/day.

Study No.: 997055

Volume #, and Page #: EDR/Pharmtox/tox/997055

Conducting Laboratory and Location: Novartis Pharmaceuticals Corporation, East Hanover, NJ

Date of Study Initiation: June 6, 1999

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 98903,

Methods: ICL670 was administered orally by gavage as suspensions in 0.5% aqueous Klucel to three groups of female rats (N= 25/group) at doses of 10, 30 and 90 mg/kg/day (10 ml/kg). A fourth group (N= 25) received an equivalent dosing volume (10 mL/ kg) of the 0.5% aqueous Klucel and served as a control. Females were treated once daily on gestation day (GD) 6 through lactation day (LD) 20. The 90 mg/kg/day dose group was terminated on LD 1 to 3 due to excessive dam/litter losses.

Doses: 0, 10, 30, 90 mg/kg/day. Doses were selected based on the results of the previous Segment I and Segment II studies in rats.

Species/Strain: Rats/Wistar Hanover (10-12 Weeks of age; Weight: 181 to 223 g)

Number/Sex/Group: 25/group

Route, Formulation, Volume: Oral, suspension in 0.5% Klucel, 10 ml/kg

Satellite Groups used for Toxicokinetics: None

Study Design: Pregnant females were treated once daily on GD6 through lactation day 20. The study design is shown below (from page 14 of the study report).

Table 3.3-1. Study design, animal allocation and test article doses

Group	Number of animals/group	Animal numbers ^a	Dose (mg/kg/day)	Concentration (mg/mL)
1 Control	25	5221-5269	0	0
2 Low	25	5275-5323	10	1.0
3 Mid	25	5329-5377	30	3.0
4 High	25	5383-5431	90	9.0

^aPSA computer assigns only odd animal numbers to females.

Parameters and Endpoints Evaluated: All females were observed twice daily for clinical signs. Food consumption and body weight determinations were conducted during the gestation and lactation periods. Following parturition, the pups were counted, sexed and weighed. All pups were observed for clinical signs and standard developmental parameters. Selected pups were observed for fertility assessments and/ or behavioral parameters. Dams were sacrificed on LD21. A gross examination of each dam and offspring was conducted at necropsy.

Results:

F₀ in-Life: Twelve of twenty-five pregnant rats at the 90 mg/kg/day were found dead during late gestation (GD19-23). Three additional animals were sacrificed moribund during late gestation (Day 20-22) and one was sacrificed moribund on Day 0 of lactation. The cause of death was not known. Piloerection was observed in 24 of 25 animals at 90 mg/kg/day. Other test treatment-related clinical signs included stains, pale, and cool to touch. The 90 mg/kg/day dose group was terminated on LD 1 to 3 due to excessive dam/litter losses. There were no mortalities in the other dose groups. Body weight and food consumption was decreased at 25 mg/kg/day. There was a statistically significant decrease in the dams surviving delivery, as 12 of 25 pregnant dams at 90 mg/kg/day group were found dead and three were sacrificed moribund before delivery.

Of the dams at 90 mg/kg/day group that survived delivery, the duration of gestation, number of implantation sites and litter size (mean number of pups delivered) were not notably different from those in the control group. However, there was a statistically significant increase in the number of stillborn pups at 90 mg/kg/day group. The following table shows the F1 cesarean data.

Parameters	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
No. of pregnant females	24	22	22	25
Females surviving delivery	24	22	22	10

Duration of gestation (mean)	21.7	21.7	21.7	21.5
With stillborn pups	1	0	1	3
Pups delivered	234	209	199	82
Implantation sites (mean)	10.3	9.9	9.9	10.9
Live pups/litter on Day 0 (mean)	9.7	9.5	9.0	7.7
Pup weight/litter on Day 0 (mean, g)	5.9	6.0	6.0	5.1
Sex ratio (male pups: total pups) on Day 0	112	116	91	34

F₀ Necropsy: Treatment-related macroscopic findings (20 of 25 dams) at 90 mg/kg/day included pale contents of the abdominal cavity in 12 of 25 dams, pale kidneys in 7 of 25 dams, yellow fluid in the small intestines in 11 of 25 dams, and dark contents in the small intestine in 1 dam. There were no remarkable findings at necropsy for the control, 10 or 30 mg/kg/day groups.

F₁ Physical Development: There were no treatment-related clinical signs in the surviving offspring. The mean initial (Day 28) and final (Day 98) body weight of control males were 82 and 393 g, respectively. The mean initial and final body weight of control females were 75 and 242 (Day 91) g, respectively. There were no significant treatment-related changes in the final body weight at 10 and 30 mg/kg/day; the body weight on lactation Day 0 was decreased at 90 mg/kg/day.

F₁ Behavioral Evaluation: Morphological and functional parameters, including righting reflex, pinna detachment, eye opening, acoustic startle, pupillary reflex, vaginal opening, and preputial separation, were evaluated. There was an apparent dose-related decrease in righting reflex (0.9 ± 0.9 , 0.6 ± 0.7 , 0.5 ± 0.7 and 0.1 ± 0.4 at 0, 10, 30 and 90 mg/kg/day, respectively) with a statistically significant difference from the control value at 30 and 90 mg/kg/day. There were no test article-related effects on the other morphological and functional parameters investigated.

Behavioral development was evaluated using an open field motor activity test and two learning/memory tests (passive avoidance and water M- maze). Open field motor activity revealed no meaningful differences for males between the 10 and 30 mg/kg/day groups and the control group. There was a trend towards increased activity for females at 30 mg/kg/day. There were no meaningful differences between the 10 and 30 mg/kg/day groups and the control group in either sex during either the learning phase or the memory phase of the passive avoidance test. Overall, there appears to be no treatment-related adverse effects on behavior.

There was a decrease in the number of trials to reach criterion for females at 10 and 30 mg/kg/day groups (the difference was statistically significant for the 10 mg/kg/day group) during the learning phase of the water M- maze test. The number of trials to reach criterion for females during the memory phase was not significantly different from that

during the learning phase for both groups. There was a statistically significant decrease in the number of errors/number of trials completed during the learning phase of the water M- maze test for females at 10 mg/kg/day group. A statistically significant decrease in the number of errors per trial during the retention phase was noted for females at 10 mg/kg/day group. There were no meaningful differences among the 0, 10 and 30 mg/kg/day groups in the results for males obtained during the learning and memory phases of the water M- maze test.

F1 Necropsy: In the litters that were delivered, the number of stillborn, the number of pups found dead, and the number of pups found with no milk in the stomach were greater at the 90 mg/kg/day group compared to those of the control group.

F₁ Reproduction: An increased incidence of renal anomalies (short/absent renal papilla in 3 of 44, 2 of 44 and 9 of 42 male animals at 0, 10 and 30 mg/kg/day and fluid-filled kidney in 0 of 44, 0 of 44 and 1 of 42 animals at 0, 10 and 30 mg/kg/day) was observed at 30 mg/kg/day F1 male parents. The incidence of these renal anomalies was not increased for males in the 10 mg/kg/day group or for females in the 10 or 30 mg/kg/day groups.

The percent of females cohoused with males that became pregnant was similar in the 0, 10 and 30 mg/kg/day groups. There were no notable differences in the mean number of corpora lutea or the mean number of implantation sites. There were statistically significant decreases in the number of total resorptions, the number of early resorptions, and the number of viable fetuses at 30 mg/kg/day. The following table (from page 38 and 39 of the study report) shows the summary of cesarean data for F1 females:

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TABLE 1.24

ICL670: AN ORAL PRE- AND POSTNATAL DEVELOPMENT STUDY IN RATS
PROJECT T-99705571

CONFIDENTIAL-TRADE SECRET

Summary of Cesarean Data

	DOSAGE	0 MG/ KG/DAY	10 MG/ KG/DAY	30 MG/ KG/DAY
Females Mated	N	24	22	22
Pregnant	N	23	20	20
%	%	96	91	91
Aborted	N	0	0	0
Premature Births	N	0	0	0
Female Mortality	N	1	0	1
Pregnant at C-section	N	22	20	19
Dams with Viable Fetuses	N	22	20	19
Dams with all Resorptions	N	0	0	0
Corpora Lutea	N	267	244	230
	MEAN	12.1	12.2	12.1
	S.D.	2.5	1.5	2.2
Implantation Sites	N	139	111	109
	MEAN	10.9	10.6	10.5
	S.D.	2.0	2.2	2.6
Preimplantation Loss	%	10.3	13.5	13.5
Postimplantation Loss	%	7.5	3.3	2.5
Dead Fetuses	N	0	0	0
%	%	0.0	0.0	0.0
Resorptions, total	N	28	7	5*
	%	7.5	3.3	2.5
	MEAN	0.8	0.3	0.3
	S.D.	1.3	0.6	0.6
Early Resorptions	N	18	7	5*
	%	7.5	3.3	2.5
	MEAN	0.8	0.3	0.3
	S.D.	1.3	0.6	0.6
Late Resorptions	N	0	0	0
	%	0.0	0.0	0.0
	MEAN	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

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TABLE 1.24

ICL670: AN ORAL PRE- AND POSTNATAL DEVELOPMENT STUDY IN RATS
PROJECT T-99705571

CONFIDENTIAL-TRADE SECRET

Summary of Cesarean Data

	DOSAGE	0 MG/ KG/DAY	10 MG/ KG/DAY	30 MG/ KG/DAY
Viable Fetuses	N	223	204	194*
	%	92	97	87
	MEAN	10.0	10.2	10.2
	S.D.	3.0	2.1	2.5
Viable Male Fetuses	N			
%	%			
Live Fetal Body Weight (g)	MEAN			
	S.D.			
Male Fetuses	MEAN			
	S.D.			
Female Fetuses	MEAN			
	S.D.			

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

Summary: In an oral (gavage) Segment III study in rats, pregnant females were administered with ICL670 at 0, 10, 30 and 90 mg/kg/day. Females were treated once daily on gestation day (GD) 6 through lactation day (LD) 20. The 90 mg/kg/day dose

group was terminated on LD 1 to 3 due to excessive dam/litter losses. There was a statistically significant increase in the number of stillborn pups at 90 mg/kg/day group. There appears to be no treatment-related adverse effects on behavior in F1 pups. There was an increased incidence of renal anomalies (short/ absent renal papilla, fluid-filled kidney) in F1 male pups sacrificed at the end of the post-weaning phase of the study at 30 mg/kg/day. However, these effects were not considered as teratogenic effects, as kidney toxicity was also observed in the neonatal rat toxicity study and were considered as nephrotoxicity associated with ICL670.

2.6.6.7 Local tolerance

Study Title: Intravenous Local Tolerance Study in the Rabbit

Key Study Findings: In a local tolerance study (0320046) in the New Zealand White rabbit, animals (n = 2/sex/group) were treated at 5 mg/kg by slow i.v. injection into the ear vein. No mortality or clinical signs were observed in this study. There was no evidence of any significant local tolerance to either ICL670 or placebo. ICL670 appeared to be locally tolerated in this study under the conditions of the experiment.

Study No.: 0320046

Volume #, and Page #: Vol. 1 of 2, page # 1

Conducting Laboratory and Location: 1

Date of Study Initiation: July 14, 2003

GLP Compliance: A compliance statement was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ICL670, Batch # 4469, 3

Formulation/Vehicle: Solution/Polyoxyl 35 castor oil (Cremophor EL, 65% w/v) and ethanol.

Methods: The purpose of this study was to examine the local tolerance of ICL670 i.v. formulation. In this local tolerance study, ICL670 i.v. solution (diluted with 5% glucose solution) was administered at 5 mg/kg by slow intravenous (i.v.) injection into the marginal vein in the direction of the left ear base to two male and female New Zealand White Albino rabbits. Placebo control group was treated with a solution of cremophor EL (65% w/v) and ethanol and diluted with 5% glucose solution as the test article solution. Saline was administered as negative control. Animals were subjected to daily

observations for clinical signs and skin reactions (erythema, edema, discoloration and necrosis) were assessed according to a numerical scoring system for 5 days after treatment. Body weights were recorded on Day 1 and 5. In addition, histopathological examinations were performed on the intravenous treatment areas and the tip of the ears.

Doses: 5 mg/kg, i.v. injection

Study Design: The study design is shown in the following table.

Study Design

Group	Animal Numbers		Treatment	
	Males	Females		
1	1-2	5-6	Saline (right ear)	Test item ID No. 132543 ¹ (left ear)
2	3-4	7-8	Saline (left ear)	Test item ID No. 131958 ² (left ear)

¹: Reference item, Identification number 132543 = Placebo

²: Test item, Identification number = 131958 = ICL670

Results: No mortality or clinical signs were observed in this study. There was no evidence of any significant local tolerance to either ICL670 or placebo. ICL670 was locally tolerated in this study under the conditions of the experiment. Based on the results of the study, it was concluded that ICL670 was locally well tolerated in rabbits when tested at 5 mg/kg i.v.

In a local tolerance study (0320046) in the New Zealand White rabbit, animals (n = 2/sex/group) were treated at 5 mg/kg by slow i.v. injection into the ear vein. No mortality or clinical signs were observed in this study. There was no evidence of any significant local tolerance to either ICL670 or placebo. ICL670 was locally tolerated in this study under the conditions of the experiment. Based on the results of the study, it was concluded that ICL670 was locally well tolerated in rabbits when tested at 5 mg/kg i.v.

2.6.6.8 Special toxicology studies

Study Title: Ames Assay with ICL670

Methods: In this exploratory study (non-GLP), ICL670

(an impurity) was tested at the following concentrations: 15, 50, 150, 500, 1500, 5000 µg/plate in TA98 and TA100 in the presence or absence of S9.

Results: Under the testing conditions, ICL670 did not show evidence of a mutagenic potential.

Study Title: 4- Week Immunotoxicity Study in Rats (Oral Gavage)

Key Study Findings: ICL670 decreased erythrocyte count, hemoglobin concentration and hematocrit associated with decreases in the reticulocyte counts compared to the respective control group. In addition, immunized controls exhibited higher reticulocyte counts than immunized animals at 60 mg/kg/day, relative to the non-immunized controls. ICL670 caused a dose-related decrease in white cell numbers for immunized males, when compared to immunized controls.

ICL670 caused a reduction in IgG in immunized males at 60 mg/kg/day, relative to immunized controls, indicating a sex-related reduction in the secondary immune response. Immunophenotyping of lymphocytes in peripheral blood indicated a reduction in relative and absolute B cell counts for immunized males at 60 mg/kg/day. A similar reduction in relative B cell counts was also observed in the spleen and mesenteric lymph nodes. Overall, oral (gavage) administration of ICL670 to rats at 6, 20 or 60 mg/kg/day for four weeks caused a lower production of IgG for immunized males at 60 mg/kg/day associated with reduced B cell counts in peripheral blood and, to a lesser extent, in the spleen and lymph nodes. These observed changes in lymphocyte subpopulations along with the IgG titers and hematology data indicated a reduction in the secondary immune response in males at 60 mg/kg/day.

Study No.: 0310048

Volume #, and Page #: EDR/Pharmtox/tox/0310048

Conducting Laboratory and Location: Novartis Pharmaceuticals AG, Basel, Switzerland

Date of Study Initiation: July 16, 2003

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ICL670, Batch No. 9923008, []

Formulation/Vehicle: Suspension in 0.5% Klucel HF

Methods: This study was conducted to examine the effect of ICL670 on the immune response to an antigen in rats. In this study, ICL670 (batch 9923008) was administered by oral gavage (5 ml/kg) to groups of rats [Wistar-Kyoto (WKY) BRL/HAN) IGS BR, n = 10/sex/group; age: 8 weeks old] at 6, 20 or 60 mg/kg/day for four weeks. Control animals similarly received the vehicle (0.5% w/v aqueous Klucel HF). Animals were immunized and subsequently challenged with Keyhole Limpet Hemocyanin (KLH) (55 µg in adjuvant/vehicle; aluminium hydroxide [1:7 v/v in 0.9% NaCl] 16 and eight days prior to necropsy, respectively. Reference animals (control and high-dose) similarly received the adjuvant/vehicle only [1:3 v/v in 0.9% NaCl). The following parameters were examined: mortality, clinical observations, body weight, food consumption, hematology, titration of KLH-specific antibodies (serum), macroscopic pathology, organ weights and histopathology, immunophenotyping of peripheral lymphocytes, splenocytes, thymocytes and mesenteric lymph nodes and toxicokinetics.

Doses: In this study, ICL670 (batch 9923008) was administered by oral gavage (5 ml/kg) to groups of rats at 6, 20 or 60 mg/kg/day for four weeks. Control animals similarly received the vehicle (0.5% w/v aqueous Klucel HF).

Study Design: The design of the study is summarized in the following table (from page 12 of the study report).

Table 1-1 Study design and test item dosages

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	Vehicle control Non-immunized	Vehicle control / KLH	ICL670 / KLH	ICL670 / KLH	ICL670 / KLH	ICL670 Non-immunized
ICL670						
Dosage (mg/kg/day)	0	0	6	20	60	60
Dosage volume (mL/kg)	5	5	5	5	5	5
KLH						
Dosage (µg/animal)	0	55	55	55	55	0
Dosage volume (mL/animal)	0.5	0.5	0.5	0.5	0.5	0.5
Number of animals/sex	10	10	10	10	10	10

Results: There were no treatment-related mortalities. Body weight gain was reduced at 60 mg/kg/day. A firm area was observed at the injections sites of all animals immunized with KLH (Groups 2 to 5). Hematological changes compared to respective control were mainly characterized by decreased erythrocyte count [Male: 95, 92 and 91 and 97% of control at 6, 20 and 60 and 60 (-KLH) mg/kg/day, respectively; Female: 100, 97, 98 and 100% of control in Groups 3, 4, 5, and 6, respectively], hemoglobin concentration (Male: 95, 93, 90 and 96% of control in Groups 3, 4, 5 and 6, respectively; Females: 99, 96, 93 and 97% of control in Groups 3, 4, 5 and 6, respectively) and hematocrit (Male: 95, 93, 90 and 96% of control; Female: 100, 97, 95 and 99% of control in Groups 3, 4, 5 and 6, respectively) and were associated with decreases in the reticulocyte counts (Male: 100, 97, 80 and 69% of control in Groups 3, 4, 5 and 6, respectively; Females: 102, 98, 91 and 93% of control in Groups 3, 4, 5 and 6, respectively). In addition, there were increases in leukocytes, neutrophils, monocytes in all immunized animals. However, ICL670 caused a dose-related decrease in white cell counts (131, 122, 120 and 120% of control in Groups 3, 4, 5 and 6, respectively) for immunized males, when compared to the immunized controls.

Histopathological changes were observed mainly in the spleen and lymph nodes of immunized animals and were characterized by an apparent increase in germinal center development and/or plasmacytosis. Increased titers of IgG and IgM were measured for animals immunized with KLH. ICL670 caused a reduction in IgG (male: 68, 89 and 6% of control, control = 95891) for immunized males at 60 mg/kg/day compared to

immunized controls, indicating a potential sex-related reduction in the secondary immune response.

Immunophenotyping of lymphocytes in peripheral blood indicated a reduction (76% of control, control = 1.72×10^6 cells/ml) B cell counts for immunized males at 60 mg/kg/day. A similar reduction in the relative B cell counts was also observed in the spleen (77% of control) and mesenteric lymph nodes (85% of control). These observed changes in lymphocyte subpopulations along with the IgG titers and hematology data indicated a reduction in the secondary immune response in males at 60 mg/kg/day.

Results of TK studies indicated that exposure was over proportional with increasing dose between 6 to 60 mg/kg/day, with C_{max} at 0.5, 1 and 2 hours post-dose at 6, 20 and 60 mg/kg/day, respectively. There was an apparent sex-related difference in exposure: at all doses, the exposure of immunized females to ICL670 and AML644 appeared to be higher than that of similarly treated males. The systemic exposure to ICL670 to males at 60 mg/kg/day appeared to be similar with and without immunization of KLH. However, for similarly dosed females, exposure to ICL670 was apparently higher with KLH-immunization than for the adjuvant alone (non-immunized). The following table (from page 35 of the study report) shows the mean TK parameters.

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Study no. 0310048Confidential
Tox report bodyPage 35
ICL670**Table 4-2 Mean toxicokinetic parameters of ICL670 in plasma (week 4)**

Group		Group 3	Group 4	Group 5	Group 6
Dosage of ICL670 (mg/kg/day)		6 + KLH	20 + KLH	60 + KLH	60 / non-immunized
Males					
t_{max}	h	0.5	1	2	2
C_{max}	µg/mL	2.09	8.33	51.6	48.0
$C_{max}/Dose$	(µg/mL)/(mg/kg/day)	0.348	0.417	0.860	0.800
AUC_{0-24h}	µg.h/mL	2.62	41.4	280	378
$AUC_{0-24h}/Dose$	(µg.h/mL)/(mg/kg/day)	0.437	2.07	4.67	6.31
Females					
t_{max}	h	0.5	1	2	1
C_{max}	µg/mL	4.07	15.2	65.1	58.3
$C_{max}/Dose$	(µg/mL)/(mg/kg/day)	0.678	0.760	1.09	0.972
AUC_{0-24h}	µg.h/mL	7.25	66.5	513	238
$AUC_{0-24h}/Dose$	(µg.h/mL)/(mg/kg/day)	1.21	3.32	8.54	3.96

Table 4-3 Mean toxicokinetic parameters AML644 in plasma (week 4)

Group		Group 3*	Group 4	Group 5	Group 6
Dosage of ICL670 (mg/kg/day)		6 + KLH	20 + KLH	60 + KLH	60 / non-immunized
Males					
t_{max}	h	-	1	2	4
C_{max}	µg/mL	-	0.299	3.64	3.30
$C_{max}/Dose$	(µg/mL)/(mg/kg/day)	-	0.0150	0.0607	0.0550
AUC_{0-24h}	µg.h/mL	-	2.15	33.4	42.9
$AUC_{0-24h}/Dose$	(µg.h/mL)/(mg/kg/day)	-	0.108	0.557	0.715
Females					
t_{max}	h	-	2	2	2
C_{max}	µg/mL	-	0.615	5.03	3.74
$C_{max}/Dose$	(µg/mL)/(mg/kg/day)	-	0.0308	0.0838	0.0623
AUC_{0-24h}	µg.h/mL	-	3.43	62.3	15.3
$AUC_{0-24h}/Dose$	(µg.h/mL)/(mg/kg/day)	-	0.172	1.04	0.255

* BLQ: analysed values below lower limit of quantification

Overall, oral (gavage) administration of ICL670 to rats at 6, 20 or 60 mg/kg/day for four weeks caused a lower production of IgG for immunized males at 60 mg/kg/day associated with reduced B cell counts in peripheral blood and, to a lesser extent, in the spleen and lymph nodes. These findings indicated a reduction in the secondary immune response of males at the highest dosage tested.

Study Title: Investigations on the Mechanism of Kidney Proximal Tubular Cell Toxicity

Key Study Findings: ICL670 caused a concentration-dependent increase in lactate dehydrogenase (LDH) leakage in LLC-PK1 cells and renal proximal tubular epithelial cells (RPTEC). For the iron complexed form of ICL670 (ICL670:Fe³⁺ = 2:1), no

significant increase in LDH leakage was observed in both cell culture systems. Treatment of LLC-PK1 cells with ICL670 in combination with equimolar iron citrate (Fe^{3+}) did not induce an increase in LDH-leakage. Incubation of kidney slices with ICL670 also increased the LDH-leakage and decreased the para-aminohippuric acid (PAH) accumulation significantly. In addition, the cellular ATP-content, ATP/ADP ratio and the energy charge (EC) were also significantly reduced. The increase in LDH leakage by ICL670 was prevented by the co-incubation with iron citrate. In addition, the decrease of cellular ATP (adenosine triphosphate)-content, ATP/ADP (adenosine diphosphate) ratio and energy charge induced by ICL670 was completely prevented by co-incubation with iron citrate. These results indicated that the iron complex of ICL670 was probably not responsible for nephrotoxicity of ICL670 and the iron chelating potential of ICL670 is probably responsible for its nephrotoxic effects.

Study No.: 971935

Volume #, and Page #: EDR/Pharmtox/tox/971935

Conducting Laboratory and Location: Novartis Pharmaceuticals AG, Basel, Switzerland

Date of Study Initiation: Not available

Date of Study Completion: April 29, 1998

GLP Compliance: A statement of compliance was included.

QA Reports: yes () no (X)

Drug, Lot #, and % Purity: ICL670. Batch number and purity data were not provided.

Formulation/Vehicle: Solution/Dimethylsulfoxide (DMSO)

Methods: In toxicity studies in mice, rats and marmosets, ICL670 caused nephrotoxicity characterized by proximal tubular changes. It was hypothesized that this kidney damage was the result of iron depletion by ICL670 rather than the consequence of intrinsic parent compound toxicity. The objective of the present study was to examine the mechanism of ICL670-induced nephrotoxicity *in vitro* using kidney cell cultures and slices. In this study, the mechanism of ICL670-induced nephrotoxicity was studied in cell cultures from kidney proximal tubule and in rat kidney slices. The pig LLC-PK1 cell line and renal proximal tubular epithelial cells (RPTEC) human renal proximal tubular epithelial cells were incubated with ICL670 for 48h at the concentrations of 0, 10, 25, 50, 100 and 150 μM . The rat kidney slices were preincubated without test compound for 1.5 h. Then the slices were incubated for 4.5 h with medium containing the different concentrations of ICL670 in DMSO in the presence or absence of 0.5 and 1 mM Fe^{3+} (0.5%) in the presence or absence of iron citrate. To evaluate the ATP-dependent uptake of para-aminohippuric acid (PAH) as an index of functional integrity of proximal tubular cells, the slices were further incubated for 30 min with 100 μM PAH containing 0.5 $\mu\text{Ci}/1.7$ ml ^3H -PAH. Afterwards the slices were washed in phosphate buffered saline (PBS), homogenized in 1 ml PBS and sonicated for 10 sec. The slice culture supernatants were collected.

The activity of LDH (lactate dehydrogenase) was determined spectrophotometrically both in the supernatants and in the cell or slice homogenates. Leakage was expressed as activity supernatant x 100/total activity (supernatant and cellular). For the determination

of the PAH-accumulation, radioactivity of [^3H]PAH in the homogenate and in the supernatant was counted using liquid scintillation counter (LSC).

Determination of ATP, ADP and AMP (adenosine monophosphate) was performed according to modified method of Gellerich et al. (*Biochem Biophys Acta* 1987. 890: 117-126). Briefly, the slices were cut in pieces and added quickly to the ice-cold buffer. After thoroughly shaking for 2 min and centrifugation for 3 min at 16000 g at 4°C the upper aqueous phase was withdrawn. It was extracted with 0.5 ml water-saturated diethyl ether and centrifuged at 4°C with 4000 g for 5 min. The lower aqueous phase was used for HPLC analysis.

Results: ICL670 caused a concentration dependent increase of LDH leakage in LLC-PK1 cells and RPTEC. LDH-leakage was statistically significantly increased in the LLC-PK1 cells with concentrations $\geq 100 \mu\text{M}$ and in the human proximal tubular primary cells at concentrations $\geq 50 \mu\text{M}$. For the iron complexed form of ICL670 (ICL670: $\text{Fe}^{3+} = 2:1$), no significant increase in LDH leakage was observed in both cell culture systems. Treatment of LLC-PK1 cells with ICL670 in combination with equimolar iron citrate (Fe^{3+}) did not induce an increase in LDH-leakage.

Incubation of kidney slices with 1 mM ICL670 also increased the LDH-leakage and decreased the PAH accumulation significantly. In addition, the cellular ATP-content, ATP/ADP ratio and the energy charge (EC) were also significantly reduced. The increase in LDH leakage by 1 mM ICL670 was prevented by the co-incubation with 0.1 mM and 1 mM iron citrate. ICL670-induced decrease in PAH-accumulation at 1 mM was almost completely reversed by co-incubation with 1 mM iron citrate. However, co-incubation with 0.1 mM iron citrate was not effective in altering ICL-induced reduction in PAH. The decrease of cellular ATP-content, ATP/ADP ratio and energy charge induced by 1 mM ICL670 was completely prevented by co-incubation with 1 mM iron citrate and partly by the addition of 0.5 mM iron citrate.

These results indicated that the iron complex of ICL670 was not nephrotoxic and the iron chelating potential of ICL670 is probably responsible for its nephrotoxic effects. The co-incubation of ICL670 with iron citrate reversed the effect on LDH leakage but not on PAH accumulation. This indicated that the decrease in PAH uptake by ICL670 was possibly not a consequence of cell death. This was thought to be due to the reduced cellular content of ATP, which was unaffected only in the presence of 1 mM iron citrate. It appeared that the iron-containing enzymes responsible for the ATP synthesis might be disturbed by the chelation of intracellular iron. Since ICL670 exerted its *in vitro* effects only in the absence of Fe^{3+} , these results indicated that that ICL670 probably exerts its effects by sequestering iron.

Study Title: Ames Assay with $\square$ J

Key Study Findings: $\square$ was found to be mutagenic in tester strain TA97 in the presence and absence of S9.

Study No.: 0212003

Volume #, and Page #: EDR/Pharmtox/tox/0212003

Conducting Laboratory and Location: Novartis Pharmaceuticals AG, Basel, Switzerland

Date of Study Initiation: Not available

Date of Study Completion: January 31, 2002

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: **C**

J Batch number CHAD0101,

C **J**. The structure of **C** **J** is shown below.

Formulation/Vehicle: Solution/Dimethylsulfoxide (DMSO)

Methods: The objective of the present study was to assess the mutagenic potential of **C** **J** (an impurity) using Ames assay using *Salmonella typhimurium* strains TA1535, TA97A, TA98, TA100 and TA102. The **C** **J** was tested at the following concentrations: first experiment (plate incorporation, using strain TA100 +/- S9 only [dose-range finding experiment]): 5, 50, 500, 5000 µg/plate; second experiment (plate incorporation): 8, 40, 200, 1000, 5000 µg/plate; third experiment (preincubation): 312.5, 625, 1250, 2500, 5000 µg/plate. The positive controls were 2-aminoanthracene (3-10 µg/plate), benzo(a)pyrene, sodium azide, 9-aminoacridine, 2-nitofluorene and mitomycin C. The vehicle (DMSO) was used as negative control.

Results: With S9, in strain TA100 signs of toxicity were seen at 5000 µg/plate, whereas **C** **J** was nontoxic in all other strains up to the highest tested dose. Without S9, the cytotoxicity was observed under plate incorporation conditions in most cases at the top dose. In case of the pre-incubation method, toxicity was generally more pronounced at 2500 or 1250 µg/plate.

In strain TA97A (frameshift mutation), in all experiments a clear and concentration-dependent increase (up to 5.86 times the concurrent solvent control value, experiment 2 + S9) in the number of revertants was seen following treatment with **C** **J**, both with or without S9. In experiment 3 in the absence of S9, no mutagenic response was found; however, there was a concentration-dependent increase in the number of revertants (up to 1.7 times the negative control). The positive controls (2-aminoanthracene) produced expected positive responses, indicating validity of the study. The results are shown in the following tables (from page 5 and 9 of the study report)

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Mutagenicity Appendix 1, Individual data

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Table 2 Second experiment (Plate incorporation)

Strain Activation	TA1535 -S9	TA1535 +S9	TA97a -S9	TA97a +S9	TA98 -S9	TA98 +S9
Concentration sp/plate						
0	22	18	169	188	39	43
	16	15	171	176	23	37
	17	15	165	160	18	56
	18 ± 3 (1.00)	16 ± 2 (1.00)	162 ± 3 (1.00)	168 ± 8 (1.00)	27 ± 11 (1.00)	45 ± 10 (1.00)
3	26	20	186	169	32	61
	21	13	170	169	33	51
	26	10	175	167	37	49
	24 ± 3 (1.33)	16 ± 5 (1.00)	177 ± 8 (1.05)	172 ± 7 (1.02)	34 ± 3 (1.25)	54 ± 6 (1.20)
40	24	15	212	189	15	75
	17	23	188	148	35	45
	31	23	180	189	32	35
	24 ± 7 (1.33)	15 ± 3 (0.94)	193 ± 17 (1.15)	175 ± 24 (1.04)	34 ± 2 (1.25)	52 ± 21 (1.16)
200	14	9	243	195	31	51
	23	10	257	185	38	47
	16	16	199	162	32	49
	18 ± 5 (1.00)	12 ± 4 (0.75)	233 ± 30 (1.39)	181 ± 14 (1.09)	33 ± 2 (1.22)	47 ± 4 (1.04)
1000	29	21	404	366	39	52
	26	18	314	306	24	31
	15	14	273	245	33	61
	24 ± 7 (1.33)	18 ± 4 (1.13)	331 ± 121 (2.37)	332 ± 75 (1.93)	32 ± 8 (1.19)	57 ± 6 (1.27)
5000	0 TNG	0	0 TNG	1002	0 TNG	46
	0 TNG	14	0 TNG	1104	0 TNG	39
	0 TNG	16	0 TNG	845	0 TNG	49
	0 ± 0 (0.00)	13 ± 4 (0.81)	0 ± 0 (0.00)	984 ± 130 (5.86)	0 ± 0 (0.00)	43 ± 5 (1.00)

Codes: TNG = absolutely no growth (no colonies, no background growth)

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Table 3 Third experiment (preincubation)

Strain Activation	TA1538 -S9	TA1535 +S9	TA97a -S9	TA97a +S9	TA98 -S9	TA98 +S9
Concentration µg/plate						
0	20	18	177	175	16	54
	18	22	215	199	22	44
	26	14	197	195	15	45
	23 ± 3 (1.00)	18 ± 4 (1.00)	195 ± 17 (1.00)	198 ± 2 (1.00)	27 ± 7 (1.00)	48 ± 6 (1.00)
312.5	23	21	252	208	43	61
	17	13	307	292	39	61
	17	15	233	280	45	43
	19 ± 3 (0.83)	16 ± 4 (0.89)	281 ± 28 (1.44)	287 ± 6 (1.45)	42 ± 3 (1.56)	55 ± 10 (1.15)
625	16	18	314	336	33	48
	13	13	316	317	41	49
	10	13	327	317	28	69
	16 ± 3 (0.83)	15 ± 3 (0.83)	320 ± 7 (1.64)	323 ± 11 (1.63)	34 ± 3 (1.25)	55 ± 11 (1.15)
1250	12 BGR	5	317	425	37	74
	20 BGR	23	352	456	22	76
	23 BGR	20	327	517	28	60
	16 ± 5 (0.75)	14 ± 10 (0.89)	332 ± 16 (1.70)	466 ± 47 (2.35)	33 ± 5 (1.07)	70 ± 5 (1.46)
2500	BGR	15	BGR	720	BGR	87
	BGR	20	BGR	635	BGR	54
	BGR	18	BGR	645	BGR	53
		18 ± 3 (1.00)		667 ± 16 (3.37)		60 ± 7 (1.25)
5000	0 TNG	14	0 TNG	811	0 TNG	16
	0 TNG	18	0 TNG	801	0 TNG	47
	0 TNG	20	0 TNG	742	0 TNG	47
	0 ± 0 (0.00)	21 ± 7 (1.17)	0 ± 0 (0.00)	791 ± 65 (3.99)	0 ± 0 (0.00)	43 ± 6 (0.90)

Codes: BGR = background growth reduced; BGO = no background growth; TNG = absolutely no growth (no colonies, no background growth)

Overall, [] produced positive results in TA97A in the presence and absence of S9.

Study Title: Chromosome Aberration Test with [] in Cultured Human Peripheral Blood Lymphocytes

Key Study Findings: [] was not found to be clastogenic in the chromosome aberration test with human lymphocytes under the test conditions used.

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Study No.: 0212201

Volume #, and Page #: EDR/Pharmtox/tox/0212201

Conducting Laboratory and Location: Novartis Pharmaceuticals AG, Basel, Switzerland

Date of Study Initiation: Not available

Date of Study Completion: March 14, 2002

GLP Compliance: A statement of compliance was not included.

QA Reports: yes () no (X)

Drug, Lot #, and % Purity: τ τ τ , Batch number CHAD0101, τ τ ;

Formulation/Vehicle: Solution/Dimethylsulfoxide (DMSO)

Methods: The purpose of this study was to evaluate the clastogenic potential of — an impurity τ τ ICL670. Cyclophosphamide (CP) and ethyl methanesulfonate (EMS) were used as positive controls in the presence and absence of S9, respectively. The concentrations for analysis of chromosome aberrations were selected based on mitotic index data. The following concentrations of — were selected for this assay:

Without metabolic activation: 322.9, 538.6, 898.5 $\mu\text{g/ml}$ (Experiment A, 20 hours treatment); 333.6, 718.8, 928.3 $\mu\text{g/ml}$ (Experiment B, 20 hours treatment).

With metabolic activation (3 hours treatment, 17 hours recovery): 538.6, 898.5, 1498.7 $\mu\text{g/ml}$, (Experiment A).

Results: ICL670 did not significantly increase chromosome aberration frequencies in this study. CP and EMS showed positive results. In conclusion, — did not appear to be clastogenic in this assay. The following tables (from page 7 and 8 of the study report) show the results of this assay.

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Table 1 Chromosome aberration analysis after treatment for 20 hours with [C] J in the absence of rat liver S9

Experiment A					Experiment B				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells		Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells
Control					Control				
DMSO/RPM	4.3 *	1.5	0.0	2.0	DMSO/RPM	7.1 *	1.5	0.0	2.4
[C] J					[C] J				
[µg/ml]					[µg/ml]				
2500.0 p	ND	ND	ND	ND	2000.0	9.9 **	ND	ND	ND
1498.7 p	16.5 **	ND	ND	ND	1548.5	9.9 **	ND	ND	ND
898.5 p	61.2 **	3.0	0.0	0.0	1199.0	27.7 **	ND	ND	ND
538.6	85.9 **	1.0	0.0	2.9	928.3	48.2 **	1.5	0.0	1.5
322.9	82.4 **	1.0	0.0	2.4	718.8	52.5 **	0.0	0.0	1.5
193.6	ND	ND	ND	ND	556.5	68.8 **	ND	ND	ND
116.0	ND	ND	ND	ND	430.9	68.8 **	ND	ND	ND
69.6	ND	ND	ND	ND	333.6	92.9 **	2.0	0.0	0.5
41.7	ND	ND	ND	ND	258.3	84.4 **	ND	ND	ND
25.0	ND	ND	ND	ND	200.0	ND	ND	ND	ND
Positive Control, EMS (µM)					Positive Control, EMS (µM)				
7.7	1.0 *	12.5	0.0	0.0	7.7	0.8 *	19.2	0.0	0.0

P precipitation
 ND not determined
 * Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.
 ** Mitotic indices as % of the controls.
 % Cells Exch.: % cells with exchanges
 % Ab. Cells: % aberrant cells (exclusive gaps)

Table 2 Chromosome aberration analysis after treatment for 3 hours with ζ in the presence of rat liver S9 and recovery for 17 hours

Experiment A				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells
Control				
DMSO/RPM	11.9 *	3.0	0.0	2.4
ζ [μ g/ml]				
2500.0 p	ND	ND	ND	ND
1498.7 p	46.6 **	2.2	0.0	5.7
898.5 p	116.0 **	2.5	0.0	2.9
538.6	113.0 **	2.5	0.0	1.0
322.9	150.4 **	ND	ND	ND
193.6	ND	ND	ND	ND
116.0	ND	ND	ND	ND
69.6	ND	ND	ND	ND
41.7	ND	ND	ND	ND
25.0	ND	ND	ND	ND
Positive Control, CP (μ M)				
55.0	13.9 *	9.0	0.0	5.7

P precipitation
 ND not determined
 * Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.
 ** Mitotic indices as % of the controls.
 % Cells Exch.: % cells with exchanges
 % Ab. Cells: % aberrant cells (exclusive gaps)

Study Title: In Vitro Cataract Study with Isolated Rat Lenses

Key Study Findings: The exposure to ICL670 for a prolonged period of time (6 days) leads to both loss of transparency (causing opacity) and glutathione (GSH) depletion, indicating an oxidative stress followed by a disruption in membrane integrity.

Study No.: 0480167

Volume #, and Page #: EDR/Pharmtox/tox/0480167

Conducting Laboratory and Location: ζ

Date of Study Initiation: August 25, 2004

Date of Study Completion: February 18, 2005

GLP Compliance: This is a non-GLP study mechanistic study.

QA Reports: yes () no (X)

Drug, Lot #, and % Purity: ICL670, Lot No. 0321001, []

Formulation/Vehicle: Solution/Dimethylsulfoxide (DMSO)

Methods: In previous toxicology studies in rats and mice, ICL670 caused bilateral cataracts. The objective of this study was to investigate the mechanism of ICL670-induced cataract formation using isolated rat lenses in the presence and absence of additional iron. In this study, the lenses (4 lenses per data point) were incubated with ICL670 at concentrations of 2 μM and 200 μM for 24 hours or 6 days in the presence and absence of additional iron as shown in the following table (from page 9 of the study report), which summarizes the conditions.

Table 3.1 Study Design

Incubation time	Concentration ICL670 (μM)	0	2	200
24 h	Normal culture conditions	4	4	4
24 h	+ iron (100 μM)	4	4	4
6 d	Normal culture conditions	4	4	4
6 d	+ iron (100 μM)	4	4	4

At the end of the incubation time, the following assays were performed as listed below. A further set of lenses to include an exposure to 20 μM concentration of ICL670 was added to the above matrix (12 lenses in total).

At the end of the incubation, the following assays were performed as listed below. A further set of lenses to include an exposure to 20 μM concentration of ICL670 was added to the above matrix (12 lenses in total).

Transparency/Opacity: The opacity images were obtained by illuminating the lenses from below with a fiber-optic ring and collecting the forward-scattered light on a [] camera using a software. The images were analyzed and quantified using a image analyzer.

LDH (Lactate Dehydrogenase)-Leakage and GSH (Glutathione): LDH and GSH were assayed using a standard assay kit.

Membrane Function: Membrane function was measured indirectly by determining sodium and potassium concentrations by []

Results:

Opacity Changes

The image analysis data revealed that, as expected, the control lenses remained transparent throughout the culture period. The greatest increases in light scatter were

observed in the presence of 200 μM . At 20 and 2 μM concentrations, ICL670 also induced small increases in the light scatter like 100 μM Fe. The presence of iron did not appear to influence the opacity changes induced by ICL670.

LDH Loss

Opacity changes are considered to be correlated with the efflux of LDH. LDH assay results showed that the greatest efflux occurred at 200 μM ICL670 at the end of the 6 day incubation period. This concentration (200 μM) of ICL670 stimulated LDH loss above control over the first 24 hours. The presence of iron potentiated this ICL670-induced LDH efflux. Iron, by itself, failed to stimulate LDH efflux.

Ion Content Changes

The K^+/Na^+ ratio in the normal rat lens was approximately 4.0. The addition of iron alone failed to alter this ratio, even after 6 days of culture. However, ICL670 caused a significant reduction in the ionic ratio after 24 hours of culture at highest tested concentration.

Glutathione Content

There was no change in the GSH level at 24 hour timepoint. However, the 6 day data revealed a concentration-dependent pattern of oxidative insult by ICL670. At 200 μM , ICL670 induced an almost total loss of GSH. The following table (from page 26 of the study report) shows the results of this study.

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Table 6-3 Quantitation of forward scattered light

Conditions	24 hours	6 days
Control	6.57 ± 0.20	7.50 ± 0.29
2 µM ICL	6.13 ± 0.23	10.75 ± 0.48
20 µM ICL	7.33 ± 0.21	14.25 ± 0.83
200 µM ICL	48.75 ± 1.25	98.50
100 µM Fe	8.13 ± 0.30	11.50 ± 1.55
2 µM ICL/Fe	7.25 ± 0.31	11.25 ± 0.25
20 µM ICL/Fe	7.17 ± 0.31	13.25 ± 0.63
200 µM ICL/Fe	50.00 ± 1.87	96.00

Lens opacity values (± SEM) after 24 hours and 6 days of culture. Opacity was measured using LensOpac/ C from digital images showing the forward scatter of light. For 200 µM ICL at 6 days exposure n = 2; for all other conditions n = 4.

Table 6-5 Measurement of LDH leakage from the lens into the surrounding medium

Conditions	24 hours	6 days
Control	-3.33 ± 1.41	1.26 ± 0.73
2 µM ICL	-3.70 ± 1.72	-0.24 ± 1.54
20 µM ICL	-1.82 ± 2.29	10.08 ± 13.38
200 µM ICL	5.80 ± 1.41	115.75 ± 4.14
100 µM Fe	-1.68 ± 1.87	-0.24 ± 0.86
2 µM ICL/Fe	-0.50 ± 1.66	1.21 ± 1.08
20 µM ICL/Fe	0.97 ± 4.69	-2.54 ± 0.59
200 µM ICL/Fe	24.70 ± 13.4	119.48 ± 6.22

LDH relative activity values (± SEM) in the culture medium of rat lenses following exposure to iron and/or ICL670. To avoid variation between plates, sample values were normalised to a positive control made by homogenising 2 freshly dissected rat lens in buffer, n = 4.

Table 6-6 Ion content as a measure of membrane changes

Conditions	24 hours	6 days
Control	3.49 ± 0.11	3.88 ± 0.03
2 µM ICL	3.85 ± 0.04	3.53 ± 0.11
20 µM ICL	3.97*	3.72 ± 0.15
200 µM ICL	1.89*	0.13*
100 µM Fe	3.52 ± 0.03	3.61 ± 0.13
2 µM ICL/Fe	3.80 ± 0.07	3.45 ± 0.37
20 µM ICL/Fe	3.72*	3.69 ± 0.25
200 µM ICL/Fe	1.99*	0.14*

The ratio of lens potassium concentration to the sodium concentration (± SEM) measured by C * n = 2; n = 4 in all other cases.

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 Appendix 2: Special information ICL670

Table 6-7 Glutathione (GSH) content of incubated rat lenses at 24 h and 6 days

Conditions	24 hours	6 days
Control	2.65 ± 0.09	1.77 ± 0.12
2 µM ICL	2.48 ± 0.13	1.04 ± 0.06
20 µM ICL	2.05*	0.88 ± 0.01
200 µM ICL	2.49*	0.13*
100 µM Fe	2.56 ± 0.15	1.70 ± 0.11
2 µM ICL/Fe	2.47 ± 0.16	1.51 ± 0.08
20 µM ICL/Fe	1.98*	0.79 ± 0.06
200 µM ICL/Fe	2.77*	0.13*

Glutathione levels (nmol mg⁻¹ lens fresh weight) in cultured rat lenses exposed to ICL670 and/or iron. * n = 2; n = 4 in all other cases.

Overall, it appeared that exposure to ICL670 for a prolonged period of time (6 days) leads to both loss of transparency and GSH, indicating an oxidative stress followed by a disruption in membrane integrity.

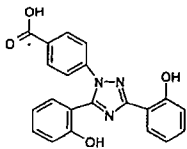
Toxicological Qualification of ICL670:

J An Impurity

is an impurity of ICL670,

The structure of and ICL-670 are shown below.

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ICL-670

As [] are generally considered as genotoxic substances, the sponsor conducted Ames assay (Study No. 0212003) and chromosome aberration assay using human peripheral blood lymphocytes (Study No. 0212201). [] was found to be positive in Ames assay in TA97A (frameshift mutation) and negative in chromosome aberration assay. Toxicological qualification of this impurity present in the Drug Substance at a maximum level of ~ppm (parts per million) is important due to its genotoxic and carcinogenic potential. [] has been reported to be weakly positive in *Salmonella typhimurium* strain TA104 at 50 µg/plate concentration []

[] In addition, [] has also been reported to be a potential carcinogen []

In this [] was tested in a carcinogenicity study in mice at 157 mg/kg/day in females and 170 mg/kg/day in males (4.7 mg/day and 5.1 mg/day, respectively, based on an average weight of 30 g). [] treatment caused smooth muscle cell tumors of the aorta and large arteries at the above dose level in both sexes. Male mice appeared to be more sensitive (42% with tumors) compared to females (14% with tumors). However, in another 26-week study [] in A/J female mouse, [] did not cause any changes in the aorta or did not increase numbers of lung tumors at 106 mg/kg/day.

Several batches of ICL670 containing this impurity have been tested in different toxicology studies. The following table shows the impurity profile of batches used in toxicology studies (from page 22 of the CMC review dated July 6, 2005).

Table 4-1 Impurity profiles of batches used in toxicology studies

Batch	Sum of [] (%)	606-21 (%)	Sum unknown [] (%)	unknown each (%)	[] (ppm)
920185 []					
97302					
98303					
992309					
992311					
0214913					

ICL670 (Batch No. 800196) containing μ parts per million (ppm) of μ (as per above table), yielded the following results: a positive result was obtained in a micronucleus test in Chinese hamster V79 cells *in vitro* (Study No. MIC127). ICL670 (Batch 97902) containing μ ppm of μ caused significant increases in the number of micronuclei in the bone marrow at 500 mg/kg in rats (Study No. 981866, Study No. 981876). However, a micronucleus test with ICL670 (Batch 98903) containing μ ppm of μ in iron overloaded rats was found to be negative at 500 mg/kg (Study No. 981878). The sponsor contended that the positive results in the *in vivo* micronucleus tests were probably due to the pharmacological mechanism of action of ICL670 and were not due to μ as it was negative in the Study 981878. It is to be noted here that the concentration of μ in the drug substance (ICL670) used for this study (981878) was less μ compared to other batches (97902: μ). The negative result in Study 981878 may be also due to less amount of this impurity in the drug substance.

Based on sponsor's maximum limit of μ ppm μ of μ in the drug substance μ , the maximum clinical dose (MCD) of μ would be μ mg/kg (base on a 50 kg body weight and a dose of 20 mg/kg/day). The calculated NOAEL (from the NOAEL of ICL670) for μ in the 26-week oral study (Study No. 987037, Batch 97902, $\mu = \mu$ mg of μ per kg of ICL670 as per above table) in rats (NOAEL for ICL670 μ mg/kg) is μ mg/kg, which offers a safety margin of 2.1. The NOAEL for μ in the 39-week oral study (Study No. 982027, Batch 98903, μ ppm or μ mg of μ per kg of ICL670 as per above table) in marmoset monkeys (NOAEL for ICL670 = μ mg/kg) is μ mg/kg, which allows a maximum dose μ of μ mg/kg or μ mg/day μ based on a 5-fold margin of safety. Therefore, sponsor's maximum limit of μ ppm is about 12.5 times more than the maximum allowable amount of μ based on toxicology study. The sponsor may be asked to reduce the level of μ to μ ppm or conduct a 2-week oral toxicity study with μ in rats to qualify this impurity in the drug substance at the proposed maximum limit of μ ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

2.6.6.9 Discussion and Conclusions

The systemic toxicity of ICL670 was adequately evaluated in a complete range of general toxicity (acute, subacute/subchronic and chronic), genotoxicity, reproductive toxicity, juvenile/neonatal toxicity and carcinogenicity studies. Repeated dose toxicity studies were conducted in rodents (mice and rats) up to 6 months duration and in non-rodents (marmoset monkeys) up to nine months duration following oral administration. The target organs of toxicity have been adequately identified in these studies. The no-observed-adverse-effect-levels (NOAEL) in mice, rats and marmoset monkeys were considered as 100 (Study No. 981060), 30 (Study No. 987037) and 40 mg/kg/day (Study

No. 982027), respectively. Overall, exposure to ICL670 in animals at NOAELs was generally less than that observed in humans at the proposed dose of 20 mg/kg/day.

The target organs of toxicity appeared to vary across species. The major target organs of toxicity revealed by the repeated dose toxicity studies in mice, rats and marmoset monkeys were kidney (in all species tested), eye (mice and rats), heart (mice and rats), gastrointestinal tract (mice and rats), and hepatobiliary tract (marmoset).

Kidney findings generally consisted of renal tubular degeneration, cytoplasmic vacuolation of cortical tubular epithelial cells and tubular necrosis across all species. In mice, tubular degeneration, tubular necrosis, regenerative changes, increased mitosis, basophilia, tubular vacuolation were generally observed at 100 mg/kg/day in non-supplemented males and at 450 mg/kg/day in iron-supplemented males and females. In the rat, nephrotoxicity was observed at 100 mg/kg/day (predominantly in non-supplemented animals) and at 180 mg/kg/day in the iron-supplemented animals. In the marmosets, renal findings were observed at ≥ 80 mg/kg/day.

Cataracts were first observed at Week 11 in the high-dose (180 mg/kg/day, 2.5 times the human exposure at 20 mg/kg/day) rats in the 26-week toxicity study. These lenticular changes were seen at all doses by the Week 26. These changes were mostly bilateral, varied in morphology from early cortical striations or vacuoles to mature cataracts. Similarly, cataracts were also observed at post partum Week 3, 4 and 9 in neonatal/juvenile rats (at 20 and 40 $\rightarrow$ 30 mg/kg/day, iron-supplemented diet, approximately 0.03 times the human exposure at *post partum* Day 75) and at Week 15 in the transgenic mice at 200 and 300 mg/kg/day in supplemented males and females, respectively. Cataracts were not observed in any monkey study.

Myocardial changes (multifocal myocytolysis in the ventricle at 600 mg/kg/day in both sexes with standard diet) were observed in the 4-week mice study. In addition, myocardial changes were also observed in the 4-week study in the rat (myocardial degeneration predominantly in males at 100 mg/kg/day with standard diet). However, cardiac effects were not observed in the marmoset.

Gastrointestinal effects (gastric erosions/ulcers, inflammation) were observed in mice (at 100 and 600 mg/kg/day in non-supplemented males and females, respectively, and at 450 mg/kg/day in iron-supplemented males and females) and rats (at 100 mg/kg/day in non-supplemented animals). In addition, inflammatory and degenerative changes in the gallbladder and bile ducts were seen in some marmosets at high doses (400 mg/kg/day in 4-Week studies and at 80 mg/kg/day in 39-week study). Similar findings were also observed in intrahepatic bile ducts in transgenic mice at 100 mg/kg/day (standard diet) at Week 4 and 26.

The target organs identified in neonatal/juvenile animals were consistent with those observed in adults. However, the results of these studies indicated an age-related difference in sensitivity to ICL670. Juvenile mice and rats appeared to be more sensitive to the toxicity of ICL670 when compared to adults. This increased sensitivity of the

neonates may be partially attributed to the age-related differences in pharmacokinetics of ICL670 between neonates and adults.

ICL670 was negative in the Ames test and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests. In addition, ICL670 was not tumorigenic in rats up to 60 mg/kg/day or p53 (+/-) transgenic mice up to 200 mg/kg/day in males and 300 mg/kg/day in females. It is to be mentioned here that the ICL670 (Batch 97902) contained _____ of _____ a genotoxic impurity, which might have contributed to the positive results in the micronucleus test at 500 mg/kg in rats (981876).

In reproductive toxicity studies in rats and rabbits by oral route, ICL670 did not appear to cause any significant adverse effects on the reproductive parameters in either sex. ICL670 did not appear to be teratogenic in rats and rabbits at the tested doses. ICL670 caused increase number of stillbirths at 90 mg/kg/day in Segment III study. There was an increased incidence of renal anomalies (short/absent renal papilla, fluid-filled kidney) in F1 rat pups sacrificed at the end of the post-weaning phase of the study at 30 mg/kg/day. Considering the nephrotoxicity of ICL670 and its ability to cross placental barrier, it appeared that these renal anomalies observed in F1 rat pups could be due to the nephrotoxicity of ICL670 and was not considered as teratogenicity.

Sponsor's maximum limit of _____ ppm of _____ a genotoxic impurity present in ICL670) was about 12.5 times more than the maximum allowable amount of _____ ppm based on toxicology study. Because of its genotoxicity and carcinogenic potential, the sponsor may be asked to reduce the level of _____ to _____ ppm or conduct a 2-week oral toxicity study with _____ in rats to qualify this impurity in the drug substance at the proposed maximum limit of _____ ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

Overall, exposure to ICL670 in animals at the NOAELs appeared to be less than that in humans at the proposed dose of 20 mg/g/day. Generally, iron supplementation appeared to attenuate target organ toxicities across all species. This could be partly due to higher drug exposure in the absence of iron supplementation. There is also an apparent sex-related difference in toxicity of ICL670 across species. Males appeared to be more sensitive to the toxicity of ICL670 when compared to females. This could again be due to observed higher drug exposure in males compared to females.

In conclusion, the non-clinical studies conducted with ICL670 adequately support its use at the intended therapeutic dosage and in accordance with the proposed product labeling.

2.6.6.10 Tables and Figures

Tables and figures were incorporated in the appropriate sections of the review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

The toxicity studies were previously listed under the section: **Studies reviewed within this submission.**

LABELING

The draft labeling of Exjade generally conforms to the format specified under 21CFR, Subpart B-Labeling Requirements for Prescription Drugs. However, the following changes should be incorporated.

1. Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Version:

Carcinogenesis/Mutagenesis/Impairment of Fertility

[

]

Evaluation: This section is in accordance with 21CFR201.57(f)(5). However, the text should be modified as suggested below.

Proposed Version: [

]

ICL670 was negative in the Ames test and chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests.

Deferasirox at oral doses up to 75 mg/kg/day (about 0.6 times the recommended human oral dose based on body surface area) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

2. Pregnancy

Sponsor's Version:

[

]

Evaluation: The text is not in accordance with 21CFR 201.57(f)(6)(i)(c). The labeling should be modified as proposed below. As the animal reproduction studies did not demonstrate a risk to fetus and there are no well controlled studies in pregnant women, the Pregnancy Category may be changed from proposed "Pregnancy category 'C'" to "Pregnancy category B".

Proposed Version:

Pregnancy. Teratogenic Effects: Pregnancy Category B.

Reproduction studies have been performed in pregnant rats at oral doses up to 100 mg/kg/day (about 0.8 times the recommended human oral dose based on the body surface area) and in pregnant rabbits at oral doses up to 50 mg/kg/day (about 0.8 times the recommended human oral dose based on the body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to ICL670. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction

studies are not always predictive of human response, ICL670 should be used during pregnancy only if clearly needed.

3. Nursing Mothers

Sponsor's Version:

Nursing Mothers

[

]

Evaluation: ICL670 is excreted in substantial amount in breast milk of rats. Following a single oral dose of 10 mg/kg of [¹⁴C]ICL670 to lactating rats, ¹⁴C radioactivity was rapidly and extensively excreted into the milk and essentially consisted of unchanged ICL670. The ¹⁴C milk/plasma ratio at 4-8 hours was about 20, indicating substantial excretion of ICL670 in the breast milk. In addition, suckling juvenile rats (PND10) were also exposed to ICL670 and/or its metabolites between one and at least 24 hours after oral administration of 10 mg/kg of [¹⁴C]ICL670 to female lactating rats. As suckling juvenile rats were exposed to the drug and its metabolites, this information should be incorporated in the labeling. The text of the labeling may be modified as suggested below.

Proposed Version: It is not known whether ICL670 is excreted in human milk. ICL670 and its metabolite were excreted in breast milk of rats following a 10 mg/kg (about 0.08 times the recommended human oral dose based on the body surface area) dose. Because many drugs are excreted in human milk, caution should be exercised when deferasirox is administered to a nursing woman.

4. Overdosage

Sponsor's Version:

OVERDOSAGE

There have been no reports of acute overdose with EXJADE® (deferasirox). Single doses up to 80 mg/kg in iron overloaded β-thalassemic patients have been well tolerated with only nausea and diarrhea noted. [

]

Evaluation: The text is in accordance with 21CFR 201.57(i).

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OVERALL CONCLUSIONS AND RECOMMENDATIONS

ICL670 (Exjade[®], CGP72670, Deferasirox) has been developed as an orally active iron chelator for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients aged 2 years and over. ICL670 is a new class of tridentate N-substituted bis-hydroxyphenyl-triazole that binds to iron (as Fe³⁺) in a 2:1 ratio. Pharmacodynamic studies with ICL670 demonstrated that ICL670 can cause removal of iron predominantly through feces. Deferoxamine is the only iron chelator approved for general use in this indication. However, patient compliance with Desferal is poor because of its short plasma half-life and poor oral bioavailability requiring daily infusions. ICL670 offers better patient compliance because of its oral route of administration. The sponsor is seeking marketing approval for ICL670 for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients aged 2 years and over.

In this NDA, the sponsor has provided the following studies: pharmacology studies; absorption, distribution, metabolism, and excretion studies in mouse, rat, rabbit, and monkeys; pharmacokinetic drug interaction studies, safety pharmacology studies; acute oral and intravenous (i.v.) toxicity studies in mouse and rat; 4-week oral toxicity studies in iron overloaded and non-iron overloaded mice; oral toxicity study in juvenile mice, 2-week intravenous and 4-week oral toxicity studies in iron overloaded and non-iron overloaded rats; oral toxicity study in juvenile rats; 2 and 4-week oral toxicity studies in iron overloaded and non-iron overloaded marmoset monkeys, 2-week i.v. toxicity study in marmoset monkeys, 39-week oral toxicity study in marmoset monkeys; genotoxicity studies: Ames assay, micronucleus assay with V79 Chinese hamster cells, chromosomal aberration test in cultured human peripheral blood lymphocytes; rat oral bone marrow micronucleus tests, induction of micronuclei in the rat liver; carcinogenicity studies: 104-week oral carcinogenicity study in Wistar Han rats and 26-week oral carcinogenicity study in p53 (+/-) transgenic mice; reproductive toxicity studies: Segment I and II and Segment III reproductive toxicity studies rat and segment II reproductive toxicity studies in the rabbit; local i.v. tolerance study in rabbits; other studies: Ames assay with κ λ , 4-week immunotoxicity study in the rat, mechanistic study to examine the mechanism of kidney proximal tubular cell toxicity, chromosome aberration assay with human peripheral blood lymphocytes with — and in vitro cataract study with isolated rat lens.

ICL670 showed higher affinity (pM = 22.5) for iron than deferiprone (pM = 19.5) and lower than that of Desferal (pM = 26.6). In bile duct cannulated rats, it was shown to be five-fold more potent than Desferal and nine-fold more potent than deferiprone. The effective oral dose of ICL670 was determined to be 32 mg/kg in rats. ICL670 treated rats showed less ⁵⁹Fe retention when compared to control. In iron-overloaded rats, ICL670 was found to be twice as effective as Desferal and 5-fold more effective than deferiprone in reducing non-heme liver iron concentration. In the iron-overloaded marmosets, at equivalent (molar) doses (112 mg/kg), the *in vivo* iron chelating efficiency of ICL670 was found to be 29% compared to 2% and 5% for deferiprone and Desferal, respectively.

ICL670 was found to be selective for iron and did not appear to induce the excretion of zinc and copper.

ICL670 was rapidly absorbed following single oral doses in mice, rats, dogs, marmosets and humans. The absolute oral bioavailability of total ICL670 (sum of ICL670 free and Fe-[ICL670]₂) ranged from 58% in mice, 32% in rats, 100% in dogs, 88% in marmosets and 73% in human. ICL670 and its iron complex Fe-[ICL670]₂ were extensively bound (>98%) to plasma proteins of all species tested including man. The volume of distribution (V_{ss}) of ICL670 at steady-state was small to moderate in mice, rats, dogs and humans (0.18- 0.82 L/ kg). In whole body autoradiography tissue distribution studies in rats, highest levels of radioactivity were seen in the gastrointestinal tract and excretory organs (liver, kidney and intestine). ICL670 and/or its metabolites crossed the blood-brain barrier to a relatively low extent. ICL670 crossed the placenta and excreted extensively into the milk in pregnant rats as unchanged ICL670. In liver microsomes from the rat, rabbit, marmoset, dog and human, oxidative biotransformation of ICL670 caused formation of mainly M1 (5-hydroxy metabolite of ICL670) and M2 (3-hydroxy), and to a minor extent M4 (5'-hydroxy metabolite) and M5 (3'-hydroxy metabolite). Formation of M1 and M2 *in vitro* was catalyzed by CYP1A1 and CYP1A2 and M4 and M5 were formed by CYP2D6. M1 and M2 were found to be about 6-fold weaker than ICL670 to complex and eliminate iron. In human and rat hepatocytes, oxidative and conjugative biotransformation of ICL670 was observed. The acyl-glucuronide conjugation of ICL670 (M3) was an important biotransformation pathway. In *in vivo* metabolic studies in mice, rats, marmosets and humans, oxidative metabolism of ICL670 was important in the rat and marmoset and was found to be low in the human and glucuronidation was also predominant in rodents, marmosets and humans. Overall, in all tested species including human, biotransformation was extensive with formation of primary (Phase I) and conjugated (Phase II) metabolites, the major metabolic pathways being hydroxylation, glucuronidation and sulfation. Irrespective of the route of administration, in all tested species, ICL670 was excreted predominantly via bile/feces. Renal excretion generally accounted for less than 12% of dose.

Acute toxicity studies (987027, 987026, 987010, 987009) were conducted in mice (CD-1) and rats (Wistar Hannover) after oral (50, 250, 500 and 1000 mg/kg in CD-1 mice; 50, 250, 500 and 1000 mg/kg in Wistar rats) and i.v. (5, 25, 50 and 150 mg/kg in CD-1 mice; 5, 25, 50 and 75 mg/kg in Wistar rats) administrations. In mice, the minimum lethal doses after oral and intravenous administration were 1000 and 150 mg/kg, respectively, in both sexes. In rats, the minimum lethal dose after oral administration was 500 mg/kg in both sexes. Following i.v. administration to rats, ICL670 was non-lethal up to 75 mg/kg in both sexes. Clinical signs in mice following oral or intravenous administrations included: discolored urine, lacrimation, ataxia, cold to touch, decreased defecation, decreased motor activity, dehydration, hunched posture, ptosis, distended abdomen, and labored breathing. Clinical signs in rats following oral or intravenous administration included decreased defecation and discolored urine.

In a 4-week toxicity study (956185) in female non-supplemented mice [(Tif:MAGf (SPF)], animals (n = 5/dose) were treated with 134 and 268 mg/kg (10 ml/kg) of ICL670

orally (gavage) for 4 weeks. The NOAEL was not established; however, 134 mg/kg/day may be considered as a tolerated dose. The target organs of toxicity appeared to be kidney (tubular degeneration at 268 mg/kg/day), thymus (atrophy at both dose levels), liver (centrilobular hypertrophy and microvacuolation at 268 mg/kg/day) and spleen (atrophy at 268 mg/kg/day dose level).

In a 4-week oral dose range-finding study (986010) in mice (C57BL/6TacBR N5 wild type) with iron-supplemented diet, animals (8/sex/group) were administered orally (gavage, 10 ml/kg) with ICL670 at 10, 30, 100, and 200 mg/kg/day, once a day for 4 weeks. The animals were given iron-supplemented diet (1680 mg/kg) throughout the study. There were no mortalities. The 100 mg/kg/day dose was identified as the NOAEL; however, 200 mg/kg/day dose was considered a tolerated dose. The target organs of toxicity appeared to be the epididymis (granulomatous, ductal dilatation, sperm stasia, oligopermia at 200 mg/kg/day), liver (decreased glycogen content at 200 mg/kg/day) and stomach (focal alteration in the glandular stomach characterized by inflammation, hypertrophy of the chief cells, atrophy of the parietal cells at all dose levels).

In another 4-week dose ranging study (0170121) in mice, iron-supplemented C57BL/6 mice (10/sex/dose) were treated with ICL-670 by oral gavage (10 ml/kg) at 100, 300 and 600→450 mg/kg/day. The animals with standard diet were treated at 100 and 600 mg/kg/day. The MTD in iron-supplemented males appeared to be 200 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day and severe iron depletion (85%) at = 300 mg/kg/day. However, in case of females, the MTD was considered as 300 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day. In case of animals with standard diet, the MTD in males appeared to be < 100 mg/kg/day based on the mortality and histopathological changes at 100 mg/kg/day. However, in case of females, the MTD was considered as 100 mg/kg/day based on the mortality at 600 mg/kg/day and absence of any significant histopathological changes at 100 mg/kg/day in females. The target organs of toxicity appeared to be the stomach (erosion, inflammation and ulcer at high dose in iron-supplemented diet and in males at 100 mg/kg/day and at 600 mg/kg/day in both sexes with standard diet), liver (focal or scattered necrosis in males at 650→450 mg/kg/day and 100 mg/kg/day in both sexes at 100 mg/kg/day animals on standard diet), lymphoid organs (lymphoid depletion, neutrophil infiltration in males at 650→450 mg/kg/day and at 100 mg/kg/day in males and 600 mg/kg/day in females), spleen (extramedullary hematopoiesis in both sexes at 650→450 mg/kg/day and at in males at 100 mg/kg/day on standard diet), kidney (tubular necrosis and regenerative changes in the cortex in both sexes at 650→450 mg/kg/day and in males at 100 mg/kg/day on standard diet) and heart (multifocal myocytolysis in the ventricle in 4 males and one female at 600 mg/kg/day animals on standard diet). Overall, it appeared that the animals fed on standard diet were more sensitive to the toxicity of ICL- 670 than animals fed on iron-supplemented diet, as evidenced by the higher MTD in iron-supplemented animals compared to non-iron-supplemented animals. In addition, there appeared to be an apparent sex-related difference in sensitivity to ICL-670. Males appeared to be more sensitive to the toxic effects of ICL-670 compared to females.

In an oral toxicity study (0480148) in juvenile mice (CD-1), animals (10/sex/time point) were treated with ICL670 by oral gavage (2 ml/kg) at 5, 15 and 35 mg/kg/day (with or without an iron-supplemented diet) for up to 5 consecutive weeks from Day 7 *post partum*. The target organ of toxicity appeared to be the liver (pigment deposition) and thymus (atrophy and necrosis). The NOAEL was considered as 5 mg/kg/day.

In a 2-week i.v. infusion toxicity study (0310064) in rats (Wistar Han, BRL/HAN) IGS BR], animals (5/sex/group) were tested intravenously at 13.5, 27 and 54 mg/kg/day for 14 days. The MTD appeared to be 27 mg/kg/day based on 10% reduction in body weight gain in males and close to 10% reduction in females relative to control. A NOAEL could not be determined, as renal toxicity was observed at all tested dose levels. The major target organ of toxicity appeared to be the kidney (tubulonephrosis, tubular basophilia/regeneration, etc.).

In a 4-week oral experimental toxicity study (971055) in rats (Wistar Hannover, n = 10/sex/group), iron-overloaded and non-iron over-loaded rats were treated orally (gavage, 5 ml/kg) with 100 mg/kg/day of ICL670. Four animals (non-supplemented) rats died on Day 11. There were no deaths in the iron-overloaded rats. The target organs of toxicity appeared to be kidney (tubular degeneration, which included renal tubular vacuolation and tubular necrosis), gastrointestinal tract in non-supplemented rats (minimal to moderate non-glandular stomach ulceration, moderate ulcerative typhlitis and slight to moderate colitis), thymus (thymus atrophy), spleen in non-supplemented rats (lymphoid atrophy, less pronounced extramedullary hemopoiesis in treated males compared to control, increased hemosiderosis in treated males), lymph node in non-supplemented rats (lymphoid atrophy in the mandibular and mesenteric lymph node), prostate (epithelial atrophy in all males), uterus in non-supplemented rats (minimal atrophy), and adrenals (cortical atrophy in non-supplemented rats). Males appeared to be more susceptible than females in non-supplemented animals.

In a 4-week oral (gavage) exploratory study (987066) in male Wistar rats (n = 10/group), animals (iron-supplemented and non-iron-supplemented diet) were treated with 100 mg/kg/day (10 ml/kg) of ICL670. Treatment-related microscopic findings were only limited to the animals fed on standard diet treated with 100 mg/kg/day of ICL670. Histopathological changes were seen (in those animals on standard diet and at 100 mg/kg/day of ICL670) in the kidney (renal cortical tubular epithelial vacuolation in 3 of 10 animals), adrenal gland (medullary vacuolation in 3 of 10 animals), stomach (gastric erosion in 2 of 10 animals), and thymus (atrophy in 2 of 10 animals).

In a 4-week oral (gavage) toxicity study (974080) in Wistar rats (16/sex/group) with a 4-week recovery period, animals received ICL670 at 10, 30, and 100 mg/kg/day (10 ml/kg). The NOAEL was established as 10 mg/kg/day. Mortality was seen at 100 mg/kg. The target organs of toxicity were kidney (renal tubular vacuolation and tubular necrosis at 100 mg/kg/day), adrenal gland (vacuolation of adrenal medulla at 100 mg/kg/day), heart [myocardial degeneration at 10 (1 of 10 female), 30 (1 of 10 male) and 100 mg/kg/day], stomach (erosion, hemorrhage, necrosis at 100 mg/kg/day), large intestine (edema, erosion, hemorrhage, inflammation at 100 mg/kg/day), large intestine (edema, erosion,

hemorrhage, inflammation at 100 mg/kg/day), small intestine (hemorrhage, epithelial hyperplasia at 100 mg/kg/day), liver (hepatocellular hypertrophy, single cell necrosis at 100 mg/kg/day), pancreas (atrophy of acinar cells and edema at 100 mg/kg/day), lymph node (erythrophagocytosis, histiocytic infiltration, lymphoid depletion, lymphoid hyperplasia at 100 mg/kg/day), spleen (atrophy, lymphoid depletion at 100 mg/kg/day), thymus (atrophy, lymphoid depletion, hemorrhage, necrosis at 100 mg/kg/day) and lung (hemorrhage and inflammation at 100 mg/kg/day). Following a 4-week recovery period, histopathological changes were noted in the heart and lymph node was still evident at 30 mg/kg/day (heart and lymph node) and 10 mg/kg/day (lymph node).

In a 26-week oral toxicity study (987037) in Wistar rats (n = 20/sex/group), ICL670 was given by oral gavage (5 ml/kg) to rats at 30, 80, and 180 mg/kg/day for 26 weeks. High dose of 180 mg/kg/day was lethal. ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (bilateral cataract at 180 mg/kg/day starting at Week 11 and at all doses at Week 26) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, the ophthalmologic toxicity (cataracts) and increased kidney weight were observed at the low dose tested (30 mg/kg/day). Therefore, maximum tolerated dose (MTD) was identified between doses of 30 and 80 mg/kg/day in males. In females, the NOAEL was considered as 30 mg/kg/day and ophthalmologic toxicity (bilateral cataracts) was observed at the dose of 80 mg/kg/day. The 80 mg/kg/day dose was considered as the MTD in females.

In an oral toxicity study (0370030) in juvenile rats (Wistar Han, iron-supplemented), animals (n = 40/sex/group) were treated with ICL670 at 0, 10, 20 and 40→30 mg/kg/day (2 ml/kg) from *post partum* day 7 through 70. Mortality was observed at 40 mg/kg/day irrespective of iron supplementation. The target organ of toxicity appeared to be the eye (bilateral cataract) and kidney (inflammatory and degenerative changes consisting of tubular vacuolation and/or necrosis and tubular dilation and/or basophilia at 40→30 mg/kg/day in males and females). The NOAEL appeared to be 20 mg/kg/day in males and 10 mg/kg/day in females. It is to be mentioned here that the NOAEL identified in the adult rat was 100 mg/kg/day. Therefore, the juvenile rats appeared to be approximately 5 to 10-fold more sensitive to the toxicity of ICL670 when compared to adults.

In a 2-week i.v. infusion study (0320068) in marmosets, marmosets (3/sex/group) were treated with ICL670 at 2, 5, and 10 mg/kg/day for 2 weeks by slow i.v. infusion (1 ml/kg). The NOAEL could not be established in this study, as histopathological changes were observed at all dose levels. The MTD appeared to be 10 mg/kg/day in the absence of any body weight change or mortality. The target organs of toxicity appeared to be the thyroid (focal lymphocytic infiltration), liver (focal necrosis, bile duct hyperplasia, focal hemorrhage) and kidney (inflammation).

In a 2-week oral gavage toxicity study (971974) in marmoset monkeys (*Callithrix jacchus*, n = 2/sex/group), animals received 0, 100, 200 and 400 mg/kg/day of ICL670 by oral gavage (5 ml/kg) for 14 days. The NOEL was established as 100 mg/kg/day. The target organs of toxicity appeared to be the kidney (cortical proximal tubular epithelial

vacuolation at 200 and 400 mg/kg/day in both sexes) and gall bladder (minimal to moderate vacuolation of the gall bladder epithelium at 200 and 400 mg/kg/day in males).

In a 2-week oral (gavage) toxicity study (971053) in iron pre-loaded marmosets, animals (10/sex/group) were treated with 400 mg/kg/day of ICL (5 ml/kg). The NOAEL appeared to be 400 mg/kg/day. There were no treatment-related clinical findings or overt toxicity findings. Histopathological alterations in the kidney (chronic interstitial nephritis characterized by lymphohistiocytic and plasma cell infiltration, tubular degeneration, atrophy associated with regeneration and glomerulonephritis), liver (diffuse iron deposition, inflammation, etc.), spleen (diffuse iron deposition) were observed in both control and treated rats. No target organs of toxicity could be identified in the absence of any treatment-related histopathological changes. ICL670 at 400 mg/kg/day appeared to be well tolerated in the iron pre-loaded marmosets under the conditions of the study.

In a 4-week oral (gavage) toxicity study (974194) in marmoset monkeys, animals (4/sex/group) received ICL670 at doses of 25, 65, and 130 mg/kg/day (5 ml/kg). Two animals/sex/group entered a 4-week recovery period following treatment. A NOAEL was established as 65 and 130 mg/kg/day in males and females, respectively. The target organs of toxicity appeared to be the kidney (vacuolar degeneration and protein casts in cortical tubules in males at 130 mg/kg), liver (vacuolation of intrahepatic bile duct cells in 1 of 4 males at 130 mg/kg/day) and gall bladder (inflammation, vacuolar hyperplasia of epithelium and fibrosis of wall of gall bladder in 1 of 4 males at 130 mg/kg/day), brain (cerebral mineralization in females at 65 and 130 mg/kg/day) and adrenal glands (adrenal cortical hemorrhage in females at 25 and 65 mg/kg/day). Protein casts seen in a single male at 130 mg/kg/day and was still evident at the end of the 4-week recovery period and a single female at 130 mg/kg/day dose level showed adrenal cortical hemorrhage at the end of the 4-week recovery period.

In a 4-week oral palatability and toxicity study (NVR030/983573 or 982041) in male marmosets, animals (n = 2/group, normal and iron-supplemented diet) were administered with ICL670 (in 0.45% w/v hydroxypropylmethyl cellulose/40% w/v polythylene glycol 400) at 200 mg/kg/day orally (gavage, 5 ml/kg). The target organs of toxicity appears to be the gall bladder (acute inflammation, increased mitotic index of epithelial cells and vacuolation of the epithelial cells), liver (neutrophil infiltration, inflammatory infiltrate in the portal areas and hemosiderin in the Kupffer cells), colon (acute inflammation and ulcer), rectum (acute inflammation and ulcer), spleen (congestion) and kidneys (dilatation of the cortical and medullary tubules and collecting ducts) in the animals given ICL670 with iron-supplemented diet.

In a 39-Week oral (gavage) toxicity study (982027) in marmoset monkeys, animals (n = 4/sex/group) were treated with ICL670 at 20, 40 and 80 mg/kg/day (5 ml/kg). Mortality was observed at 80 mg/kg/day. Histopathologic changes in dead or moribund animals were observed in the gall bladder (acute inflammation and generalized degeneration), hepatic bile duct (vacuolation) and kidneys (vacuolation and degeneration of the renal cortical tubules and dilatation of the medullary tubules and Bowman's space). The 40 mg/kg/day dose may be considered as the NOAEL.

ICL670 was negative in the Ames test and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. It was positive in 1 of 3 *in vivo* oral rat micronucleus tests.

In a 104-week oral (gavage) carcinogenicity study (017022) in Wistar Hannover rats, ICL670 was administered orally via gavage (5 ml/kg) as a suspension in aqueous 0.5% Klucel, to 5 groups of rats (50/sex/group) at daily doses of 0 (standard feed), 0 (feed supplemented with iron), 25→15 (feed supplemented with iron), 50→30 (feed supplemented with iron) and 100→60 mg/kg/day (feed supplemented with iron). Doses were decreased on Day 29 of the study based upon the recommendations of ExecCAC. Doses were selected based upon the toxicity endpoint (MTD) from a 26-week oral (gavage) toxicity study in rats at 30, 80 and 180 mg/kg/day with a 4-week recovery period [Study 983037]. No test article-related mortality or moribundity was evident upon histopathological assessment. At study termination, 72-84% survival was observed in the males and 58-68% survival was observed in the females. There were no statistical differences in survival between the control groups and ICL670-treated dose groups. There were no test article-related effects noted during palpable mass examination. There were no apparent significant treatment-related changes between the incidence and severity of non-neoplastic lesions when compared to control. There was no treatment-related increase in tumor incidence for either male or female and there was no statistically significant dose-response trend in tumor rates.

In a 26-week carcinogenicity study (0270117) in p53 (+/-) transgenic mice, ICL670 was administered orally via gavage (10 ml/kg) as a suspension in aqueous 0.5% Klucel to 25 male p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 20, 100 and 200 mg/kg/day (feed supplemented with iron), and 30 mg/kg/day (standard feed) and to 25 female p53 heterozygous mice/group at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 30, 100 and 300 mg/kg/day (feed supplemented with iron) and 100→60 mg/kg/day (standard feed, dose was decreased on Day 50). Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine (400 mg/kg/day, oral, gavage) and served as a positive control. ICL670 was also administered orally via gavage as a suspension in aqueous 0.5% Klucel, to p53 wild type mice (25/sex/group) at daily doses of 0 (feed supplemented with iron), 0 (standard feed), 200 mg/kg/day (males, feed supplemented with iron), or 300 mg/kg/day (females, feed supplemented with iron). The doses for the current study were selected based on MTD and as per CDER ExecCAC recommendations (ExecCAC meeting minutes dated August 21, 2001, September 17, 2002 and November 12, 2002, Appendix-3, 4, and 5, respectively). Treatment-related lenticular changes of bilateral mature cataracts were observed during Weeks 15 and 26 in iron supplemented p53 heterozygous mice at doses of 200 mg/kg/day (males) and 300 mg/kg/day (females) and in one wild type female mouse at 300 mg/kg/day. There were no treatment-related neoplastic findings either in the p53 heterozygous mice or wild type mice in the presence or absence of iron supplementation. The positive control, p-cresidine, produced effects,

which included proliferative changes in the urinary bladder (transitional or squamous cell carcinoma and papilloma, 40-64% in males and 20-24% in females) and liver (hepatocellular adenoma, 4%). Treatment-related non-neoplastic findings were observed in the eye (cataract), kidney (increased basophilic tubules; vacuolar changes in the tubular epithelium), liver (biliary hyperplasia and inflammation), glandular and non-glandular stomach (erosion/ ulceration, hyperkeratosis in females) and skeletal muscle (degeneration/regeneration). Wild type animals generally shared the same repertoire of test article-related changes as the equivalent, iron-supplemented p53 heterozygotes. Generally, among the p53 heterozygous mice, cataract, gastric hyperkeratosis and muscle degeneration were mainly confined to iron-supplemented mice which tolerated higher doses of the test article than did non-supplemented mice. However, biliary hyperplasia, hepatic periportal inflammation and increased basophilic kidney tubules were confined generally to non-supplemented mice.

In a segment I fertility and reproductive performance study (987102) in Wistar Han rats (n = 25/sex/group), male and female rats received ICL670 by oral gavage (10 ml/kg) at doses of 7.5, 25, and 75 mg/kg/day. Male rats were treated with ICL670 for 28 days prior to mating, during the mating period and until terminal sacrifice (between study days 50 and 54). Female rats received ICL670 for 14 days prior to mating, during the 2-week mating period and through gestation day 6 (GD6). There were no adverse effects on mating, fertility or reproductive parameters of rats including early embryonic development at any of the tested dose levels.

In a Segment II reproductive toxicity study (Study No. 987055) in Wistar Han rats, pregnant female rats (n = 25/group) received ICL670 by oral gavage (10 ml/kg) at doses of 10, 30, and 100 mg/kg/day from GD6 to GD17. Mortality was observed at 100 mg/kg/day with associated gastrointestinal lesions in two animals on GD17 and GD18. There were no treatment-related changes in reproductive parameters including the mean number of viable fetuses, early and late resorptions, post-implantation loss. In the 100 mg/kg/day group, one fetus each from three litters had external malformations: one fetus had orofacial malformations (proboscis, protruding tongue, and maxillary micrognathia); one fetus had encephaly and another fetus had omphalocele. Omphalocele was also observed in one control fetus. These malformations were not considered to be treatment-related in the absence of a dose response and occurrence in the control animal. There appeared to be no treatment-related visceral malformations in fetuses. There were no meaningful skeletal malformations among the groups. A significant number of skeletal variations were observed at 100 mg/kg/day (full supernumerary ribs, supernumerary lumbar vertebra and centrum); however, there were no dose-response relationships and none of these effects was considered to have any impact on the survival. ICL670 was not considered to be teratogenic in rats at the tested doses.

In a segment II teratogenicity study (987056) in New Zealand rabbits, pregnant female rabbits (n = 20/group) received ICL670 by oral gavage (5 ml/kg) at 5, 20, and 50 mg/kg/day from GD7 to GD20. ICL670 appeared to have no effect on the reproductive parameters. Multiple thoracic vertebra malformations (hemivertebra, malpositioned and fused vertebra, misaligned, fused and misshapen centrum as well as hemicentrum, etc.)

were observed at 20 mg/kg/day; however, there were no skeletal malformations at 5 or 50 mg/kg/day. These skeletal malformations observed at 20 mg/kg/day appeared to be incidental because of the lack of a dose-response relationship and seems not related to treatment. There were no meaningful differences in skeletal variations observed among the groups. However, none of these variations would be expected to have an impact on survival. ICL670 was not considered to be teratogenic in rabbits under the conditions of the study.

In an oral (gavage) Segment III study (997055) in Wistar Han rats, pregnant females (25/group) were administered with ICL670 at 0, 10, 30 and 90 mg/kg/day (10 ml/kg) from GD6 through lactation day (LD) 20. The 90 mg/kg/day dose group was terminated on LD1 to LD3 due to excessive dam/litter losses. There was a statistically significant increase in the number of stillborn pups at 90 mg/kg/day group. ICL670 had no adverse effects on behavior of F1 pups. There was an increased incidence of renal anomalies (short/absent renal papilla, fluid-filled kidney) in F1 pups sacrificed at the end of the post-weaning phase of the study at 30 mg/kg/day. However, these effects do not appear to be teratogenic, as treatment-related kidney toxicity was also observed in neonatal rat study.

In a local tolerance study (0320046) in the New Zealand White rabbits, animals (n = 2/sex/group) were treated at 5 mg/kg by slow i.v. injection into the ear vein. No mortality or clinical signs were observed in this study. ICL670 appeared to be locally tolerated in this study under the conditions of the experiment.

The potential of ICL670 to alter immunological activity was examined in an immunotoxicity study (0310048] in the rat at 6, 20 and 60 mg/kg/day for 4 weeks. ICL670 showed some effects on immune parameters, which included a dose-related decrease in white blood cells and lower IgG titers, and a decrease in absolute peripheral and tissue B cells in males at 60 mg/kg/day. It is to be mentioned here that in a previous study in juvenile mice (Study No. 0480148), ICL670 at 35 mg/kg/day also caused decreases in B and T-cell populations in the spleen and decreases in T-cell populations in the thymus. Overall, these findings indicated a potential of ICL670 to cause reduction in the secondary immune response.

The mechanism of ICL670-induced nephrotoxicity observed in mice, rats and monkeys was further studied in cell cultures of porcine or human kidney proximal tubule cells and in rat kidney slices (971935). ICL670 caused cell membrane damage as evidenced by increased leakage of lactate dehydrogenase (LDH). This effect was not observed in the presence of Fe^{3+} . In rat kidney slices, para-aminohippuric acid (PAH) uptake, a parameter of functional integrity of proximal tubular cells, and ATP content were also decreased in the presence of ICL670. These effects were almost completely blocked by iron. Based on these results, it appeared that ICL670-induced nephrotoxicity could be partly attributable to iron depletion.

The mechanism of ICL670-induced cataracts in mice and rats was further investigated *in vitro* using isolated rat lens (0480167). ICL670 exposure to rat lenses for 6 days resulted

in the loss of both transparency and glutathione without any effect on the K^+/Na^+ ratio suggesting that the stress induced by ICL670 is probably oxidative in nature followed by a disruption in membrane integrity.

Toxicological qualification of [redacted] an impurity of ICL670, ICL670, was conducted by using batches of ICL670 in different toxicity studies containing this impurity. Sponsor's maximum limit of [redacted] ppm for [redacted] was determined to be 12.5 times more than the maximum allowable amount of [redacted] ppm based on NOAEL for [redacted] in toxicology studies. As [redacted] genotoxic and possesses carcinogenic potential, the presence of this impurity in the drug substance above qualification threshold [redacted] ppm raises a significant concern. The sponsor may be asked to reduce the level of [redacted] to [redacted] ppm or conduct a 2-week oral toxicity study with [redacted] in rats to qualify this impurity in the drug substance at the proposed maximum limit of [redacted] ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

The systemic toxicity of ICL670 was adequately evaluated in a complete range of general toxicity (acute, subacute and chronic), genotoxicity, reproductive toxicity, juvenile/neonatal toxicity and carcinogenicity studies. The preclinical program of ICL670 appeared to be successful in identifying the target organs of toxicity. ICL670 was genotoxic in one *in vivo* micronucleus test. However, ICL670 was not tumorigenic in 26-week study in p53 (+/-) transgenic mice and 104-week study in rats. In fertility and reproductive performance study in rats, ICL670 did not cause any adverse effect. It was also not teratogenic in rats or rabbits. Therefore, from a preclinical standpoint, this NDA may be approved.

The labeling of ICL670 conforms to the format specified under 21CFR, Subpart B. However, the suggested changes described in the text, should be incorporated.

Conclusions: From a preclinical standpoint, this submission meets the guidelines and satisfies the criteria for marketing authorization of ICL670 and appears to be safe for the proposed use.

Unresolved Toxicology Issues: The sponsor may be asked to reduce the level of impurity [redacted] in the drug substance to [redacted] ppm or conduct a 2-week oral toxicity study with [redacted] in rats to qualify this impurity in the drug substance at the proposed maximum limit of [redacted] ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

Recommendations:

1. From a preclinical standpoint, this NDA may be approved.
2. The sponsor may be asked to reduce the level of [redacted], an impurity in the drug substance, to [redacted] ppm or conduct a 2-week oral toxicity study with [redacted] in rats to qualify this impurity in the drug substance at the proposed maximum limit of [redacted] ppm.

ppm as a postmarketing commitment as per ICH Q3A Guidance on Impurities in New Drug Substances (February 2003).

Suggested Labeling: The sponsor may be asked to modify the proposed label of Exjade as suggested in the text of this review.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

cc:

Original NDA
HFD-180
HFD-160/CSO/PM/AKacuba/DMIHP
HFD-180/Dr. Choudary
HFD-180/Dr. Chakraborti
HFD-160/Dr. ALaniyonu
HFD-160/Dr. Robie Suh/DMIHP
HFD-048/Dr. Viswanathan

R/D Init. J Choudary: 8/24/05

APPENDIX/ATTACHMENTS

Appendix-1: Exec CAC meeting minutes dated June 5, 2001 for rat carcinogenicity study, page: 282

Appendix-2: List of neoplastic and non-neoplastic lesions for rat carcinogenicity study, page: 286

Appendix-3: Executive CAC meeting minutes dated August 21, 2001 for mouse carcinogenicity study, page: 316

Appendix-4: Executive CAC meeting minutes dated September 17, 2002 for mouse carcinogenicity study, page: 321

Appendix-5: Executive CAC meeting minutes dated November 12, 2002 for mouse carcinogenicity study, page: 326

Appendix-6: List of neoplastic and non-neoplastic lesions for mouse carcinogenicity study, page: 332

APPENDIX-1

Exec CAC Meeting Minutes dated June 5, 2001 for Rat Carcinogenicity Study

Appears This Way
On Original

Executive CAC

June 5, 2001

Committee:

Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Ke Zhang, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Ke Zhang

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 58,554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

Background:

ICL-670, an iron chelator, is currently under clinical investigation for transfusion-dependent β -thalassemia. Sponsor submitted a protocol for 2-year carcinogenicity study in rats along with the report of a 26-week oral toxicity study in rats in this submission.

Rat Carcinogenicity Dose Selection:

The proposed route of administration is oral gavage. Wistar rats (50/sex/group) will be given ICL-670 at 25, 50, and 100 mg/kg/day. There are two control groups. Animals in control 1 group will receive a standard diet. Animals in control 2 group and the three treatment groups will be fed with an iron supplemented diet (~1680 ppm). Toxicokinetic parameters will be conducted in 10 animals/sex in each group during weeks 4 and 52. The treatment duration is 104 weeks. The following observations will be conducted: clinical signs of toxicity, body weight, food consumption, ophthalmoscopy, clinical pathology including CBC and serum iron, renal parameters, tissue iron analysis (liver and kidney), organ weights, gross and histopathology. The sponsor did not clearly indicate whether the histopathology will be conducted in all animals from all groups.

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Page 2

The dose selection was based on the results of the 26-week oral toxicity study in rats. In this study, ICL-670 was given to rats by oral gavage at 30, 80, and 180 mg/kg/day. The high dose of 180 mg/kg/day was lethal and exceeded the MTD. ICL670 produced following toxicity at 80 mg/kg/day or higher: ophthalmologic toxicity (cataracts) and renal toxicity (increases in blood urea nitrogen and creatinine, cytoplasmic vacuolation of cortical tubular epithelium in males). In males, ophthalmologic toxicity (cataracts) and increased kidney weights were observed at the low dose tested (30 mg/kg/day). Therefore, the MTD was identified as between doses of 30 and 80 mg/kg/day in males. In females, the dose of 30 mg/kg/day was a no effect dose and ophthalmologic toxicity (cataracts) was observed at the dose of 80 mg/kg/day. The dose of 80 mg/kg/day was close to the MTD in females. In the 4-week dose ranging studies, the dose of 100 mg/kg/day was lethal. It appears that the high dose of 100 mg/kg/day proposed by sponsor is excessive.

The sponsor stated that a clinically effective dose is expected between 5 and 40 mg/kg. The AUC_{0-24hr} of ICL670 in humans following a single oral dose of 20 mg/kg was 388 $\mu\text{mol}\cdot\text{h}/\text{l}$ or 144.5 $\mu\text{g}\cdot\text{h}/\text{ml}$. The AUC_{0-24hr} of ICL670 in rats following an oral dose of 80 mg/kg/day (431 $\mu\text{g}\cdot\text{h}/\text{ml}$ in males or 314 $\mu\text{g}\cdot\text{h}/\text{ml}$ in females at week 12) is approximately 2-3 folds of that (144.5 $\mu\text{g}\cdot\text{h}/\text{ml}$) in humans after 20 mg/kg.

Executive CAC Recommendations and Conclusions:

The Committee did not concur with the sponsor's proposed doses. The Committee recommended doses of 60, 30, and 15 mg/kg/day due to concerns for toxicity at higher doses and a concern that the high dose proposed by the sponsor is above the MTD.

It is recommended that the histopathological examination be conducted in all animals from all groups. If the sponsor plans histopathological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances:

- (a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups,
- (b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group,
- (c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI

IND 58,554
Page 3

76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,

(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\n
/Division File, HFD-180
/Dr. Choudary, HFD-180
/Dr. Zhang, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

APPENDIX-2

List of Neoplastic and Non-Neoplastic Lesions for Rat Carcinogenicity Study

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Table 3.1
 TOL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 1
 PLACE 1996 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day	100-50 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day	100-50 ng/kg/ day
CARDIOVASCULAR SYSTEM											
HEART											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Cardiomyopathy.	10	12	14	11	11	25	29	27	27	24	
Hyperplasia, endocardium.	35	37	36	38	39	21	21	22	22	23	
Bacteria.					2			1		1	
HEAVE HEART TUMOR (H)		1						1		2	
endocardium.								1			
Inflammation, acute.									1		
Fibrosis, pericardium.											
AORTA											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Mineralization.	45	50	50	50	50	50	50	55	48	50	
1									1		
ARTERY											
Periarteritis.		(1)		(1)							
1				1							
DIGESTIVE SYSTEM											
SALIVARY GLAND											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	
Atrophy, acinar, focal.	45	43	49	49	48	46	45	48	48	48	
Basophilic, focal.	1				1						
Inflammation	1	1				1					
Metaplasia, squamous.	1		1		2		1	1			

(H) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TOL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 2
 PLACE 1996 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day	100-50 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day	100-50 ng/kg/ day
DIGESTIVE SYSTEM											
SALIVARY GLAND											
Atrophy, acinar, focal.	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	
Hemorrhage.								1	1	1	
TONGUE											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
50	50	50	50	50	50	50	50	50	50	50	
ESOPHAGUS											
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)	
Inflammation, acute.	50	50	50	49	50	50	50	48	48	49	
Perforation.								2			
Hemorrhage.								1			
STOMACH											
Within normal limits	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Congestion, focal.	35	41	17	40	43	44	38	41	38	41	
Cyst, glandular.		1			1						
Cyst, nonglandular.		1	1				2	2	4	2	
Dilatation, gland, glandular stomach.	1										
Edema, nonglandular.			1	1				2	2		
Edema, glandular.			1	1	2	1	4	2	1		
Excess, glandular stomach.					2		2	1			
Hemorrhage, glandular stomach.		1			2		1	4		1	
Hyperplasia, mucosa, glandular stomach, focal.					1					1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1
 TOLVENE: 104-week oral (gavage) carcinogenicity study in mice
 Study No. 917022

23-AUG-04 12:14
 Page: 3
 PLACES 2805 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	5 mg/kg/day	25-30 mg/kg/day	50-60 mg/kg/day	100-160 mg/kg/day	0 mg/kg/day	5 mg/kg/day	25-30 mg/kg/day	50-60 mg/kg/day	100-160 mg/kg/day
DIGESTIVE SYSTEM											
STOMACH		(50)	(50)	(45)	(50)	(50)	(20)	(50)	(50)	(40)	(50)
Infiltrate, lymphocyte, glandular stomach, focal.						1					
Inflammation, nonglandular			1	1			1	1	1	1	
Mislocalization	1	1		1				1	1	1	
Fleer, nonglandular				1				2	2	2	1
Vascularization, epithelium			1					2	2	2	
Vascularization, epithelium, limiting ridge	2	4	0	4	1	2	2			2	
Inflammatory cell infiltrate, submucosa							1				
Hyperplasia, focal, epithelium, nonglandular				1							
Ulcer, glandular								1		1	
LEIOMYOSARCOMA (M)							1				1
Inflammation								2			
Haemorrhage, limiting ridge, focal											1
Haemorrhage, glandular stomach, multifocal										1	
Inflammation, nonglandular, acute, focal											1
Hyperplasia, epithelium, nonglandular										1	
Edema, glandular stomach					1				1	1	
SPONGE CELL CARCINOMA (M)											1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL578: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 01922

25-AUG-04 12:14
 Page : 4
 PLACEE 2002 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-335 mg/kg/day	100-360 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-335 mg/kg/day	100-360 mg/kg/day
DIGESTIVE SYSTEM											
ESOPHAGUS SARCOMA (M)		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
DUODENUM											
Within normal limits		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Dilatation, segmental		45	50	46	47	50	50	49	50	49	50
Diverticulum		1		1	1						
Inflammation		1		1	1			1			
Mineralization				1						1	
Fibrosis				1							
Mineralization, focal					1						
Infiltrate, eosinophil								1			
GENITRUM											
Within normal limits		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Dilatation		50	47	50	50	49	50	45	45	45	50
Enlargement			1			1			1		
Fibrosis, focal			1								
Inflammation						1			1		1
Bacteria, serosa, acute, focal										1	
Fibrosis								1			
Peritonitis									1		
ILEUM											
Within normal limits		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
		50	49	50	47	50	50	45	50	50	50

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 2.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 019022

23-MAR-04 12:14
 Page: 5
 BLACS 2000 V2.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25- 75 mg/kg/ day	50- 100 mg/kg/ day	100- 500 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25- 75 mg/kg/ day	50- 100 mg/kg/ day	100- 500 mg/kg/ day
DIGESTIVE SYSTEM											
ILEUM											
Dilatation.	(50) 50	(50) 1	(50)	(50)	(50)	(50) 1	(50)	(50)	(50)	(50)	(50)
COLON											
Within normal limits	(50) 50	(50) 49	(50) 47	(50) 49	(50) 50	(50) 50	(50) 50	(50) 49	(50) 50	(50) 49	(50) 49
Dilatation.		1									
Infiltrate, eosinophil.			1			1					1
Inflammation.											
Emulsion.											
Infiltrate, eosinophil.											
Aggregation, macrophage.											
Increased.											
RECTUM											
Within normal limits	(50) 50	(50) 50	(50) 49	(50) 50	(50) 50	(50) 50	(50) 50	(50) 49	(50) 50	(50) 49	(50) 49
Edema.											
SARCOMA [M]			1								1

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL679: 104-week oral [gavage] carcinogenicity study in rats
 Study No. 017022

23-MAY-04 12:14
 Page: 6
 BAAEP 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-215 ng/kg/ day	50-210 ng/kg/ day	100-260 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-215 ng/kg/ day	50-210 ng/kg/ day	100-260 ng/kg/ day
DIGESTIVE SYSTEM											
MESENTERY	(1)		(2)	(3)			(2)	(1)		(1)	
Accessory spleen.				1			1			1	
Fat necrosis.				1							
Hemorrhage, focal.				1							
PANCREAS	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits.	37	38	37	36	37	38	33	34	40	43	
Alteration, basophilic, focal.	1	1		1	2			1			
Atrophy, acinar.	5	4	7	7	4	5	11	6	5	3	
Dilatation, duct.	1	2	2	3	1		1	1	1		
Edema.						1					
Fibrosis, perivascular.						1					
Hypertrophy, islet cell, focal.	1		1	1			1	2			
Inflammation.	3	6	1	4	7	4	4	5	4	4	
ISLET CELL ADENOMA [B].	1		2		1	1					
ISLET CELL CARCINOMA [M].	1		1	3	1						
Periarteritis.						2		1			
Hemorrhage.									1		

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICH679: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 7
 BLA658 2005 V1.DOC

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	5 mg/kg/ day	25-35 mg/kg/ day	50-350 mg/kg/ day	100-360 mg/kg/ day	0 mg/kg/ day	5 mg/kg/ day	25-35 mg/kg/ day	50-350 mg/kg/ day	100-360 mg/kg/ day
HEPATOBLILIARY SYSTEM											
BILE DUCT - EXTRAHEPATIC Dilatation.		(2) 2	(2) 2	(2) 2	(4) 4	(2) 2	(3) 3	(2) 2		(2) 2	(1) 1
LIVER											
Within normal limits		(50) 10	(50) 10	(50) 6	(50) 8	(50) 14	(50) 7	(50) 3	(50) 6	(50) 11	(50) 12
Angiectasis.		1	1	2	2	1	2	2	3	1	1
Cyst, biliary.		1	2	2	2	3	5	4	2	1	5
Degeneration, cystic.					3	1			1		
Dilatation, bile duct, focal.					2						
Dilatation, sinusoid, focal.		15	7	7	2	8	7	8	4	3	3
Fibrosis				1		1					1
Focus, acidophilic.		14	13	15	16	12	6	7	11	4	5
Focus, basophilic.		1	1	1	1	1	2	1	1	1	1
Focus, basophilic, tigroid.		22	16	18	14	16	31	33	24	22	26
Focus, clear cell.		1		1		1					
HEPATOCELLULAR ADENOMA [B].		1		1	1	2	2	2	3		
HEPATOCELLULAR CARCINOMA [M].			1		2	2		1	1		
Hernia, diaphragmatic.			1	1					1		
Hypertrophy, bile duct.		7	5	10	8	7	15	11	10	11	11
Hypertrophy, hepatocellular, focal.				1							
Infiltrate, lymphocyte, focal.			2			1	1	1	1		2
Inflammation		5	2	8	2	7	1	2	5	8	4
Mineralization, focal.					1				1		

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TEL76: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 019222

23-AUG-04 12:14
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 PLACEE 2500 VI.D00

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25-315 mg/kg/ day	50-330 mg/kg/ day	100-600 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25-315 mg/kg/ day	50-330 mg/kg/ day	100-600 mg/kg/ day
HEPATOBIILIARY SYSTEM											
LIVER	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Mitotic figures, increased.						1				2	
Necrosis, focal.	1	1	1					1	1		
Necrosis, centrilobular.			1							1	
Necrosis, single cell, focal.		1								1	
Pigment, hepatocellular, Kupfer cell.	1	2				1	1	3	4	1	
Tension focus.	1	1	1	2	2	2	3	3	2	1	
Vacuolation.	3	2	5	2	2	3	4	1	1	2	
Vacuolation, focal.	4	1	6	4	4	1	1	1	4	2	
Vacuolation, centrilobular.	1	1	2	1	1	1	1	1	2	1	
Extrasinusoidal hepatopossis.	2	3	2	3	2	3	3	3	2	2	
Necrosis.								1			
Fibrosis, periportal.									1		
Deformity, focal.								1			
CHOLECYSTOMA (B)											
Pigment, macrophage.			1					1			
ENDOCRINE SYSTEM											
ADRENAL	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Nidus normal limits	27	22	25	31	29	10	5	16	17	12	
Angiectasis, cortex.	3			1	5	14	22	24	27	32	
Angiectasis, medulla.											
Atrophy, cortex.					1			2			
Cyst.		1								1	

(B) Benign tumor
 Figures in [] represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 101676: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017622

23-APR-04 12:14
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 PLACES 2000 01.006

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25- 50 ng/kg/ day	50- 100 ng/kg/ day	100- 500 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25- 50 ng/kg/ day	50- 100 ng/kg/ day	100- 500 ng/kg/ day
ENDOCRINE SYSTEM											
ADRENAL											
Hypertrophy, cortex, focal.	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	
Hypertrophy, cortex, cystic, focal.	13	13	17	10	9	10	15	14	9	11	
Infiltrate, lymphocyte, focal.					1						
PHROCHROMOCYTOMA (B)	3	2	1	1				1			
Vasculature, cortex, focal.	3	5	5	3	4	1					
CORTICAL ADENOMA (B)				1	2	2	1		1	1	
Hyperplasia, focal, cortex.	1				1						
Hyperplasia, focal, medulla.			1							1	
PHROCHROMOCYTOMA (M)							1				
Paraneoplasia.							1				
Necrosis, cortex.							1	1	2	2	
Hypertrophy, cortex.							1	1	3		
Hemorrhage.										1	
HEMANGIOENDOTHELIOMA (M)							1				
Fibrosis, cortex.							2		1		
Extramedullary hematopoiesis, increased.	3	1		2	1		5	2	2	3	
Infiltrate, lymphocyte.		1									
PARATHYROID											
Within normal limits.	(42)	(50)	(48)	(49)	(48)	(46)	(47)	(47)	(47)	(48)	
Hyperplasia.	42	42	43	44	43	46	43	45	47	47	

(B) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TOLCER: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017622

23-AUG-04 12:14
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 PLACES 2000 01.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-25 mg/kg/day	50-50 mg/kg/day	100-500 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-25 mg/kg/day	50-50 mg/kg/day	100-500 mg/kg/day
ENDOCRINE SYSTEM											
PARATHYROID		(40)	(50)	(40)	(49)	(48)	(44)	(47)	(47)	(47)	(49)
Hyperplasia, focal.		2	7	6	6	5		3		(47)	1
ADENOMA (N).		2	1	1	1			1		2	
PITUITARY		(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits		15	21	17	21	16	15	12	17	14	17
ADENOMA (N), pars distalis.		17	15	19	15	20	28	28	22	29	23
ADENOMA (N), pars intermedia.		1	1	2			1				
CARCINOMA (N), pars distalis.						1			2		
Cyst.		10	4	5	4	7		2	1	1	2
Hamartoma					1	1					
Hyperplasia, focal, pars distalis.		3	8	11	9	6	6	9	6	6	7
Hyperplasia, focal, pars intermedia.		1				1		1			
THYROID		(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)
Within normal limits		27	26	34	25	24	17	21	20	31	24
F-CELL ADENOMA (N).		3	3	7	8	9	5	13	6	7	5
C-CELL CARCINOMA (N).			1		2				1		
Cyst, follicular.		2			2	1	1		1		
Cyst, ultimobranchial.			1			1			3		3
Ectopic thymus.						1					
Hyperplasia, C-cell, diffuse.		3	2		5	2	2	5	5	5	6
COLLUSION ADENOMA (N).		4	5	4	2			1	1	1	1

(N) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TCL67D: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

27-AUG-04 12:14
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 BLAZE 2500 V1.002

ORGAN/TISSUE FINDING	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day	100-360 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day	100-360 mg/kg/day
ENDOCRINE SYSTEM											
THYROID											
Hyperplasia, focal, C-cell.	(50) 8	(50) 6	(50) 5	(50) 4	(50) 12	(45) 5	(50) 3	(50) 8	(50) 7	(50) 8	
Hyperplasia, focal, follicular.	5	5		3	3	1	2			2	
Inflammation								1	1	1	
FOLLICULAR ADENOCARCINOMA [M]	1									1	
GENITOURINARY SYSTEM											
NERVE											
Within normal limits						(50) 45	(50) 47	(50) 48	(50) 50	(50) 48	
Cyst							1				
LEIOMYOSARCOMA [M]							1				
Hypertrophy						1	1				
Hyperplasia, epithelium.						1				1	
Aggregation, granular cell.						1					
POLYP [M]							1				
EPITHELIMIDES											
Within normal limits	(50) 46	(50) 42	(50) 43	(50) 42	(50) 45						
Adenoma.	2	7	6	5	5						
Fibroma.				1							
Fibrosis.		1									
Granuloma, sperm.		2	1	2	1						

[M] Benign tumor
 [N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically.

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Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 12
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ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS											
	TREATMENT	Males					Females					
		0 mg/kg/day	25-315 mg/kg/day	50-330 mg/kg/day	100-660 mg/kg/day	0 mg/kg/day	25-15 mg/kg/day	50-330 mg/kg/day	100-660 mg/kg/day	0 mg/kg/day	25-15 mg/kg/day	50-330 mg/kg/day
GENITOURINARY SYSTEM												
EPIDIDYMIDES												
Hematestis, vascular.	(50)	(50)	(50)	(50)	(50)							
Inflammation	1		1	1								
NEPHROTICOMA [M]												
Inflammation, chronic, focal.			2	2								
KIDNEY												
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
ADENOMA [E]	14	15	13	18	28	5	8	7	6		12	
Basophilic tubules.			1	3	3	1	1				3	
Calculi.	2		1	1		1	2				1	
Cast, proteinaceous.			1	1				4	2		4	
Chronic progressive nephropathy.	28	27	31	22	11	7	12	12	8		8	
Cyst.	2	1	2	2	1		1	2			1	
Dilatation, tubular.		4		1	1	1		1	3		2	
Hemorrhage.		1										
Hyaline droplets.	1		1	1				1	1			
Hydronephrosis.		5		1	2			1	1			
Hyperplasia, epithelium, pelvis.					1							
Hyperplasia, epithelium, pelvis, focal.	1		1			2		2	2	5	2	
Infarct, chronic.	1											
Inflammation	1	1	5	3	4	1		3	4		2	

[E] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 019022

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 PLACES 2096 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-215 mg/kg/day	50-230 mg/kg/day	100-260 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-215 mg/kg/day	50-230 mg/kg/day	100-260 mg/kg/day
GENITOURINARY SYSTEM											
KIDNEY	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
LIPOMATOSIS (M).				1							
Mineralization, focal.	2	5		1		23	38	19	22	24	
Mineralization, epithelial, pelvis, focal.	3	2	1	2	2	34	25	34	23	35	
Necrosis, medulla.		1									
Necrosis, papillary.	1				1			1		1	
Necrosis, tubular.			1	2		4	4	9	4	4	
Parakeratosis.				1	2					4	
Cyst, tubular.			1	1				1	3	2	
Cystitis.	1					2	2	1	2	1	
Hypertrophy, focal, tubular.		1						1			
Bactera.									1		
Fibrosis.						1					
TRANSITIONAL CELL PAPILLOMA (M).	1										
TRANSITIONAL CELL CARCINOMA (M).				1							
OVARY						(50)	(50)	(50)	(50)	(50)	
Within normal limits.						35	45	41	41	38	
Cyst.						5	4	5	7	10	
Cyst, hemorrhagic.											
ADENOCARCINOMA (M).											
THYROID CELL TUMOR (M).								1			

(M) Benign tumor

(M) Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 917022

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 PLACES 2500 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-115 ng/kg/ day	50-130 ng/kg/ day	100-160 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-115 ng/kg/ day	50-130 ng/kg/ day	100-160 ng/kg/ day
GENITOURINARY SYSTEM											
OVARY											
TUBULAR CELL TUMOR (B)						(50)	(50)	(50)	(50)	(50)	
LUTEAL CELL TUMOR (B)							1				
Hemorrhage							1	1			
Hyperplasia, tubulostromal									1		1
Atrophy									1		1
PROSTATE											
Within normal limits	(50)	(50)	(50)	(50)	(50)						
ADENOCARCINOMA (B)	17	71	25	14	35						
Inflammation	18	10	22	14	12						
Secretion reduced	1	1	1	1	1						
Hyperplasia, focal	4	2	1	2	3						
ADENOMA (B)	1	1	1								
SEMINAL VESICLE											
Within normal limits	(50)	(50)	(50)	(50)	(50)						
ADENOCARCINOMA (B)	48	43	42	45	44						
Dilatation	1										
Inflammation	1	1	1	1	3						
Secretion reduced		5	5	4	5						
TESTIS											
Within normal limits	(50)	(50)	(50)	(50)	(50)						
Atrophy, seminiferous tubule	41	37	37	32	42						
	7	5	5	10	8						

(B) Benign tumor

(B) Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TCE670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACES 2005 01.000

ORGAN/TISSUE FINDING	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25- 15 mg/kg/ day	50- 30 mg/kg/ day	100- 60 mg/kg/ day	0 mg/kg/ day	25- 15 mg/kg/ day	50- 30 mg/kg/ day	100- 60 mg/kg/ day	
GENITOURINARY SYSTEM											
TESTIS	(50)	(50)	(50)	(50)	(50)						
Edema.		1	2	1	1						
Granuloma, sperm.		1									
INTRAGESTIVAL CHILL TUMOR (E).	2	3	1	1							
Metaplasia, fosal.				1							
Inflammation, sperm.	1	1			2						
Hyperplasia, focal, interstitial cell.				1							
URINER		(1)			(1)			(1)	(1)		
Dilatation.		1			1			1	1		
Inflammation, acute.								1	1		
URINARY BLADDER	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(48)	(50)	(50)	
Within normal limits	47	47	49	49	47	49	50	46	47	49	
Calculi.	1				1				1		
Dilatation.	1	2		1	1				2		
Hemorrhage.		1				1			1		
Hyperplasia, transitional cell.	1				2			1	1	1	
Inflammation						1			1	1	
Edema.	2				2	1			1	1	
UTERUS						(50)	(50)	(50)	(50)	(50)	
Within normal limits						36	33	32	35	37	

(E) Borderline tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 BLACRE 2000 01.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25- 315 mg/kg/ day	50- 310 mg/kg/ day	100- 360 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25- 315 mg/kg/ day	50- 310 mg/kg/ day	100- 360 mg/kg/ day
GENITOURINARY SYSTEM											
UTERUS											
ADENOCARCINOMA [M]						(50)	(50)	(50)	(50)	(50)	
CHORIOCARCINOMA [M]							2	2	1	2	
Cyst						7	7	0	4	6	
Dilatation						3	3	1	2	1	
ENDOMETRIAL STROMAL POLYP [B]						3	5	5	7	3	
ENDOMETRIAL STROMAL SARCOMA [M]						1	1	2			
Inflammation						1	1			1	
Thrombosis							1				
Necrosis							1				
Hyperplasia, endometrial						2	1	1	2		
Hyperthecae							1				
MESENCHYMAL SARCOMA [M]						1					
Adenomyosis							1				
VAGINA											
Within normal limits						(50)	(50)	(50)	(50)	(50)	
Aggregation, granular cell						44	28	43	37	49	
GRANULAR CELL TUMOR [B]						2	6	2	2	1	
POLYP [B]						2	2	2	1	2	
Inflammation							1		2	1	
Prolapse							1		1		
LEIOMYOSARCOMA [M]						1					

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
ICL676: 104-week oral (gavage) carcinogenicity study in rats
Study No. 019022

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PLACES 2005 01.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25- 15 ng/kg/ day	50- 30 ng/kg/ day	100- 60 ng/kg/ day	0 ng/kg/ day	25- 15 ng/kg/ day	50- 30 ng/kg/ day	100- 60 ng/kg/ day	
GENITOURINARY SYSTEM											
VAS DEFERENS											
Granuloma, sperm.		(1) 1									
HEMOLYMPHATIC SYSTEM											
SPLEEN MARROW	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	43	40	46	44	46	44	41	45	41	45	
Granulopoiesis, increased.	3	4	1	2	2	1	7	3	4	4	
Hamatopoiesis, increased.	2	4		1		3	5		3		
Hamatopoiesis, decreased.		1		1	1		1		2	1	
Hyperplasia, lymphoid, focal.						1					
Fibrosis.	2	1	2	1				1			
LYMPH NODE	(1)	(1)		(1)							
Edema, sinus.		1		1							
Hemorrhage, mediastinal.	1										
LYMPH NODE - BRONCHIAL	(46)	(47)	(47)	(47)	(49)	(45)	(45)	(46)	(46)	(46)	
Within normal limits	30	27	38	40	40	44	45	39	43	44	
Accumulation, erythrocyte.	1	1		2	2		2	2	1	1	
Edema, sinus.	5	6	5	5	6		1	1	1	1	
Infiltrate, neutrophil.						1					
Pigment, macrophage.						1			1		
Bacteria.								1			
Aggregation, macrophage.		2	1		2	1		1			
Increased.								1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 1CL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 317822

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 BLACH 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25->10 mg/kg/ day	50->10 mg/kg/ day	100->50 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25->10 mg/kg/ day	50->10 mg/kg/ day	100->50 mg/kg/ day
HEMOLYMPHATIC SYSTEM											
LYMPH NODE - INGUINAL											
Ecstasia, sinus.		(3)									
7											
LYMPH NODE - NODULAR	(50)	(50)	(50)	(50)	(49)	(49)	(50)	(50)	(50)	(49)	
Within normal limits	34	41	42	46	45	48	44	45	44	43	
Ecstasia, sinus.	3	8	3	4	2	4	2	2	4	3	
Hemorrhage.	1										
Hyperplasia, lymphoid.	1		1					1	1	1	
Hyperplasia, plasma cell.	2				2		2	1	1	2	
Euplasia, lymphoid.								1			
Accumulation, erythrocyte.			1						2		
LYMPH NODE - MEDIASTINAL	(4)	(7)	(6)	(7)	(2)	(5)	(3)	(2)	(3)		
Accumulation, erythrocyte.	2	2	1	1	1	3	1	1	3		
Ecstasia, sinus.	2	7	4	6	1			1			
1		1									
HEMANGIOSARCOMA (M)											
Infiltrate, neutrophil.						1					
Inflammation, acute.								1			
Hyperplasia, plasma cell.								1			
LYMPH NODE - MESENTERIC	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)		
Within normal limits	35	34	35	32	31	26	20	24	21		
Accumulation, erythrocyte.			4	3			2				
Ecstasia, sinus.	2	3	6	3	3		4	1	3		
Fibrosis.				1			1				
1				2			1				
HEMANGIOSARCOMA (M)											

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TGL676: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

29-AUG-04 12:14
 Page: 15
 BLAZE 2626 V1.006

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-330 mg/kg/day	100-360 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-330 mg/kg/day	100-360 mg/kg/day
HEMOLYMPHATIC SYSTEM											
LYMPH NODE - MESENTERIC	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Histiocytosis, sinus.		1	1								
Hyperplasia, lymphoid.		2				1	1	2	1	1	
Hyperplasia, plasma cell.						1					
Pigment, macrophage.						1					
Aggregation, macrophage.						20	20	23	25	32	
Increased.	10	5	10	10	22	20	23	23	25	32	
LYMPH NODE - PANCREATIC	(1)	(1)	(2)	(1)				(1)			
Accumulation, erythrocyte.		1	2								
Ecchymosis, sinus.		1	1	1							
Fibrosis.											
Pigment, macrophage.								1			
LYMPH NODE - DUODENAL		(1)									
Ecchymosis, sinus.		1									
LYMPH NODE - RENAL		(2)	(1)								
Width normal limits.		1									
Ecchymosis, sinus.		1	1								
Histiocytosis, sinus.		1									
LYMPH NODE - SUBLINGUAL	(1)	(5)		(1)	(5)	(1)	(1)	(1)	(1)	(1)	
Ecchymosis, sinus.	1	4		1	4	1	1	1	1	1	
HEMANGIOENDOTHELIOMA [M].											
Hemorrhage.											

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-84 12:14
 Page: 20
 BLACS 1986 V1.90B

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25->15 mg/kg/day	50->30 mg/kg/day	100->60 mg/kg/day
HEMOLYMPHATIC SYSTEM											
LYMPH NODE - SUBMANDIBULAR											
Histiocytosis, sinus.		(1)	(5)		(1)	(5)	(1)	(1)	(1)	(3)	(1)
Hyperplasia, lymphoid.			1							2	1
Hyperplasia, plasma cell.			1			2				1	
Necrosis, focal.						1					
THYROID											
Within normal limits		(45)	(50)	(48)	(48)	(50)	(50)	(50)	(48)	(49)	(50)
Atrophy, lymphoid.		43	40	35	35	47	21	25	21	25	24
Cyst.											
Hyperplasia, epithelial.		2	5	8	6	4	24	21	26	15	19
Hemorrhage.				1	1	1				1	
Hyperplasia, [B].		2	1		1	3	2	4	5	5	6
Hyperplasia, [M].			1					1		1	1
Hyperplasia, focal.				1	2		3		2		1
Hemorrhage, focal.							1				
Hyperplasia, lymphoid.									1		
Ectopic parathyroid.			1								
SPLEEN											
Within normal limits		(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Depletion, lymphoid.		45	42	43	43	45	38	23	30	35	39
HEMOLYMPHATIC SYSTEM [M].					1	1					
Pigment.			1	2	1	1				2	

[B] Benign tumor
 [M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically.

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Table 3.1
 12L678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 PLACEB IPGS W1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	5 ng/kg/ day	25-50 ng/kg/ day	50-100 ng/kg/ day	100-500 ng/kg/ day	0 ng/kg/ day	25-50 ng/kg/ day	50-100 ng/kg/ day	100-500 ng/kg/ day	
HEMOLYMPHATIC SYSTEM											
SPLEEN		(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(49)	
Extramedullary hematopoiesis, increased.		4	6	2	6	2	10	17	8	5	
Periarteritis.								1			
Necrosis, focal.										1	
Hyperplasia, lymphoid.										2	
Nodules.									1		
Congestion, focal.										1	
Accessory spleen.									1		
Cyst, capsular.										1	
Hyperplasia, histiocytic, focal.				1					1		
SYSTEMIC/ANY SITE		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits		48	49	48	50	50	49	50	49	50	
HISTIOCYTIC SARCOMA (M).											
LYMPHOMA (M).		2	1	1			1		1		
ENDOCRINE SYSTEM											
THYROID GLAND											
Within normal limits						(50)	(49)	(49)	(48)	(48)	
Inflammation.						27	22	28	29	31	
Hypertrophy.						29	27	29	19	17	
Hyperplasia, focal.									1		
SQUAMOUS CELL CARCINOMA (M).										1	

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 1.1
 KCL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AD2-04 12:14
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 PHACEE 2009 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 ng/kg/ day	0 ng/kg/ day	25-325 ng/kg/ day	50-330 ng/kg/ day	100-560 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-310 ng/kg/ day	50-330 ng/kg/ day
INTEGUMENTARY SYSTEM										
MAMMARY GLAND										
Within normal limits	(48)	(47)	(50)	(46)	(47)	(50)	(50)	(50)	(50)	(50)
ADENOCARCINOMA (M)	47	44	48	44	46	30	26	28	32	30
ADENOMA (B)						8	2	2	3	1
FIBROADENOMA (B)						4	12	8	3	1
FIBROSIS (B)						1				8
Galactosia		1	1	2		5	4	5	6	2
Hyperplasia		1			1	8	13	8	10	11
Inflammation									1	
Hyperplasia, with acypla						2		1	1	3
ADENOLIPOMA (B)						1				
PROSTATIC GLAND										
Within normal limits	(50)	(49)	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Dilatation	18	15	18	24	24	24				
Hemorrhage, chronic			1							
Inflammation	32	30	30	25	25					
Mineralization, focal				1						
SKIN										
Within normal limits	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Atrophy, follicular	19	23	41	31	49	39	49	49	44	42
BASEAL CELL TUMOR (B)	1	6	1	2	2	4	4	7	4	4
Cyst, inclusion		2	1	2	1	1	1	1		1
Ulcer, surface, focal		1								

(B) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 1CL679: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
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 PLACES 2006 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 ng/kg/ day	5 ng/kg/ day	25-515 ng/kg/ day	50-530 ng/kg/ day	100-560 ng/kg/ day	0 ng/kg/ day	25-515 ng/kg/ day	50-530 ng/kg/ day	100-560 ng/kg/ day
INTESTINE										
SKIN										
FIBRINOMA [B]		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
FIBROSARCOMA [B]		1	2			1				1
Fibrosis			1		1	1		1		1
HEMANGIOENDOTHELIOMA [B]					1					
Hemorrhage					1					1
Hyperplasia, sebaceous gland, focal				1						
Inflammation		2	4	3	1	3	3	2		
KERATOCARCINOMA [B]		3	5	2	2	1			1	
LEIOMA [B]		1	1					1	1	
LIPOMA [B]		2	1	1			1			
Pigment, focal				1					1	
Ulceration		1		1		1		1		
NERVE SHEATH TUMOR [B], cystic			1				1			
SEBACEOUS ADENOMA [B]		1								
SEBACEOUS CARCINOMA [B]										1
SQUAM CELL CARCINOMA [B]		1					1	1		
Acanthosis						1	2	1	3	
NERVE SHEATH TUMOR [B]		1				1				
LEIOMYOSARCOMA [B]										

[B] Benign tumor
 [B] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL670: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 917922

27-JUL-04 12:14
 Page: 24
 BLAKE 7850 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males						Females		
		0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day	100-60 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-15 ng/kg/ day	50-30 ng/kg/ day
MUSCULOSKELETAL SYSTEM										
BONE			(1)	(1)	(1)	(1)	(1)			(1)
Hyperostosis.										
OSTEOBLASTOMA (M).				1		1				
Bacteria, focal.									1	
Inflammation.									1	
Endosteitis.									1	
BONE - FEMUR/TIBIA		(48)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Within normal limits.		48	50	50	50	49	48	50	50	49
Chondrocytropy, joint.						1				
BONE - STERNUM		(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)
Within normal limits.		49	49	50	50	50	50	49	50	50
Hyperostosis.		1	1							
Regeneration.										
chondrocytic, focal.										
DIAPHRAGM						(1)	(1)	(1)	(2)	
Bacteria.									1	
Inflammation.						1	1	1	1	
Fibrosis, focal.								1		
MUSCLE		(49)	(50)	(49)	(50)	(50)	(49)	(50)	(49)	(50)
Within normal limits.		48	48	48	48	48	47	48	48	48
Atrophy.		1	1		1	1	1	1	1	1
HEMANGIOBLASTOMA (M).									1	1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
1CL678: 104-mesh oral (gavage) carcinogenicity study in rats
Study No. 917622

23-ADX-04 12:14
Page : 25
ELRCES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS										
	TREATMENT	Males					Females				
		0 ng/kg/day	0 ng/kg/day	25->15 ng/kg/day	50->30 ng/kg/day	100->60 ng/kg/day	0 ng/kg/day	0 ng/kg/day	25->15 ng/kg/day	50->30 ng/kg/day	100->60 ng/kg/day
MUSCULOSKELETAL SYSTEM											
MUSCLE	(48)	(50)	(48)	(50)	(50)	(48)	(50)	(50)	(48)	(50)	
Hemorrhage, focal.							1				
Inflammation	1	1						1			
Necrosis	1										
Pigment, focal.							1				
HEMORRHAGE [M].								1			
Bacteria								1			
GRANULAR CELL TUMOR [B].						1					
Foreign body present.								1			
Thrombus									1		
FIBROSARCOMA [M].				1							
NERVOUS SYSTEM											
BRAIN	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	
Within normal limits	44	41	15	43	42	15	35	40	37	38	
Compression	3	7	11	5	6	5	10	8	10	10	
Edema			1								
GRANULAR CELL TUMOR [B].			1							1	
Hemorrhage			1								
Hydrocephalus	3	4	8	3	1	5	6	5	10	7	
Mineralization, focal.		1		1			1				
Mineralization, vascular.											
meninges											
GLIOBLASTOMA [M].	1			1					1		

[B] Benign tumor
[M] Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
KLE670: 104-week oral (gavage) carcinogenicity study in rats
Study No. 017022

25-AUG-04 12:14
Page : 26
BLANKS 2806 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENT OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-25 mg/kg/day	50-30 mg/kg/day	100-50 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-25 mg/kg/day	50-30 mg/kg/day	100-50 mg/kg/day
NERVOUS SYSTEM											
BRAIN	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
ASTROCYTOMA (M)					1						1
GLIOMAS (M)					1						1
NEURONAL CELL TUMOR (M)			2						2		
NERVE - SCIATIC	(48)	(50)	(49)	(50)	(49)	(45)	(50)	(50)	(49)	(50)	(50)
Within normal limits	48	48	48	50	49	48	50	50	48	50	50
Degeneration	1	2				1			1		
SPINAL CORD	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits	17	14	19	19	18	25	23	29	24	27	27
Compression					1						
Degeneration, white matter	7	5	5	3	2	6	10	4	7	3	
Degeneration, spinal nerve	33	34	31	29	31	25	24	21	26	22	
RESPIRATORY SYSTEM											
LUNG	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(42)	(49)	(49)
Within normal limits	25	30	40	27	26	15	34	19	37	26	26
Accumulation, macrophage, foamy	15	16	5	14	22	8	14	7	11	14	14
EMPHYSEMA (M)					1		1	1			1
Bronchial/alveolar congestion	2		1	1		1					2
Edema	1										

(M) Benign tumor
(M) Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 TELSTO: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 27
 PLACES 2500 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/ day	0 mg/kg/ day	25- 25 mg/kg/ day	50- 50 mg/kg/ day	100- 50 mg/kg/ day	0 mg/kg/ day	0 mg/kg/ day	25- 25 mg/kg/ day	50- 50 mg/kg/ day	100- 50 mg/kg/ day
RESPIRATORY SYSTEM											
LUNG		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(47)	(49)
Foreign material present, bronchial.						1					
Hemorrhage.		4	3	1	3			1	1	1	3
Infiltrate, granulocyte, perivascular.		1	1			1					
Infiltrate, lymphocyte, multifocal.				1							
Inflammation.		4	3	3	3	1	3	4	2	3	3
Myopathy, vein.		1									
Bacteria.									1		
Hyperplasia, focal, bronchioalveolar.			2			1	2			1	2
Pigment, macrophage.					1		1			1	
NASAL CAVITY										(1)	
Accumulation, blood, lumen.										1	
TRACHEA		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Mucosa normal limits.		50	50	50	49	50	50	50	50	49	49
Inflammation.					1						
GAST TISSUE											
PAN		(2)	(2)	(1)		(2)	(4)	(6)	(5)	(6)	(5)
Fat necrosis.			2	1		1	4	2	4	6	5
Hemorrhage.						1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically.

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Table 3.1
 IRL678: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page : 28
 PLACES 2800 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 ng/kg/ day	0 ng/kg/ day	25-215 ng/kg/ day	50-230 ng/kg/ day	100-260 ng/kg/ day	0 ng/kg/ day	0 ng/kg/ day	25-215 ng/kg/ day	50-230 ng/kg/ day	100-260 ng/kg/ day
SOFT TISSUE											
FAT - EPCOM Within normal limits	(1) 1										
FAT - PERIOVARIAN Hamartoma.						(1) 1					
SPECIAL SENSE ORGANS											
EAR											
Within normal limits			(1) 1	(1) 1					(1) 1		
Chondrodysplasia, pinna.											1
EYE											
Within normal limits	(50) 43	(50) 42	(50) 47	(50) 41	(50) 43	(50) 42	(50) 41	(50) 42	(50) 45	(49) 44	
Absent.			1								
Atrophy, retina.	6	5	2	7	5	7	7	6	6	4	
Cataract.		2									
Cyst, retrobulbar.							2				
Hemorrhage					1	1					
Hyalinitation, iris/ciliary body.		1		1	1	1		1			
Inflammation					1	1			1		1
Mineralization, cornea	1			1			2				1
No abnormality detected.											
Synechia.		1									
Hyperplasia, epithelion, cornea.											1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICLE79: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 617022

23-AUG-04 12:14
 Page : 29
 BLAZE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS									
		Males					Females				
		0 mg/kg/day	0 mg/kg/day	25-215 mg/kg/day	50-230 mg/kg/day	100-260 mg/kg/day	0 mg/kg/day	25-215 mg/kg/day	50-230 mg/kg/day	100-260 mg/kg/day	
SPECIAL SENSE ORGANS											
EYE		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Fluid, proteinaceous, subconjunctival chamber.										1	
Dilatation.											1
Mucous bulbi.											
MANDIBULAR GLAND		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Within normal limits	40	33	33	33	34	33	35	27	30	31	31
Atrophy, acinar.	1	5	6	4	2	12	8	13	13	13	13
Inflammation	9	14	12	15	14	11	9	11	15	9	
Cyst.						1					
Hyperplasia, focal.											
LACRIMAL GLAND		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within normal limits	22	18	13	17	20	35	41	46	40	43	43
Inflammation	14	12	14	9	10	4	4	2	5	1	
Metaplasia.	26	26	34	30	27	2	7	2	4	6	
Atrophy, focal.		1				1			2		
TYMPAL GLAND			(2)								(2)
CARCINOMA (M).			2								1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 1.1
 ICL676: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

23-AUG-04 12:14
 Page: 30
 BLACER 1999 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day	100-360 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES										
ABDOMINAL CAVITY Inflammation, mild, acute. Fat necrosis.							(1) 1			(1) 1
FORELIMB No abnormality detected. nail.				(2) 2		(1) 1				
HINDLIMB Within normal limits Edema. Eosinophilia. HEPATOBLASTOMA [B]. Inflammation Inflammation, mild, acute. No abnormality detected. nail. Pododermatitis. No abnormality detected.	(21) 1 1 1 1 10 15	(15) 6 12	(10) 1 1 1 9 9	(12) 1 1 1 8 5	(21) 1 1 1 4 16	(10) 3	(3) 3	(5) 7	(5) 3 1	(9) 4 5 1
MEDIASTINUM NERVE SHEATH TUMOR [B]. cystic. Bacterial. Inflammation, acute.					(1) 1		(1) 1		(2) 2	

[B] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.1
 ICL676: 104-week oral (gavage) carcinogenicity study in rats
 Study No. 017022

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 BLACER 1999 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS								
		Males					Females			
		0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day	100-360 mg/kg/day	0 mg/kg/day	0 mg/kg/day	25-315 mg/kg/day	50-310 mg/kg/day
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES										
MEDIASTINUM Foreign body present.					(1)		(1)		(2) 1	
TAIL CHORDOMA [B].		(1) 1								

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-3

Executive Committee Meeting Minutes Dated August 21, 2001 for Mouse
Carcinogenicity Study

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Executive CAC**August 7, 2001**

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Charles Resnick, Ph.D., HFD-110, Alternate Member
Karen DavisBruno, Ph.D., HFD-510, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 26-week carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL 670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias : This drug is currently under Phase I clinical trial. The sponsor has submitted a protocol and dose selection for the 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 30, 100, 300, and 600 mg/kg/day doses.

P53 Mouse Carcinogenicity Dose Selection:

The sponsor has proposed to conduct a 26-week oral (gavage) carcinogenicity study in p53 (+/-) transgenic mice at 30, 100, 300, and 600 mg/kg/day doses. The sponsor's dose selection was based on the results of the previous 4-week oral dose range-finding study in C57BL/6 mice with iron-supplemented diet. In the 4-week oral dose ranging study, the animals were orally administered ICL 670 at 10, 30, 100, and 200 mg/kg/day. There was no mortality. At Week 4, the mean body weight gain in males was reduced, but not in a dose-related manner, at all dose levels (21.8%, 39.2%, 30.5%, and 60.9% reduction at 10, 30, 100, and 200 mg/kg/day, respectively) when compared to body weight gain in controls. The body weight gain in females at Week 4 was also reduced (21.8%, 21.8%, 26.1%, and 17.4% at 10, 30, 100, and 200 mg/kg/day, respectively) compared to control but not in a dose-related manner. The body weight gain data appeared to be erratic, exaggerated (0.9 to 1.9 g weight gain compared to 2.3 g in control) and did not follow any dose-related pattern. These data were not considered reliable to use as the basis of dose selection for the proposed carcinogenicity study for females. The 100 mg/kg/day was identified as the NOEL. At 200 mg/kg/day, there was decreased glycogen

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content in the liver of all treated males. Treatment-related histopathological changes were observed in the stomach (focal alteration in the glandular stomach characterized by inflammation, hypertrophy of the chief cells, atrophy of the parietal cells) of males and females at all dose levels in a low incidence; however, the changes were not dose-related.

ICL 670 was found to be negative in the Ames assay and did not show any clastogenic potential in the chromosome aberration test with human peripheral blood lymphocytes. The sponsor conducted three in vivo rat bone marrow micronucleus tests by oral route. In the first experiment in non-iron-supplemented rats, ICL 670 showed a marginal but statistically significant increase ($0.14 \pm 0.05\%$, $0.22 \pm 0.11\%$, and $0.27 \pm 0.11\%$ at 50, 158, and 500 mg/kg, respectively, vs. $0.14 \pm 0.06\%$ in control) in the number of micronucleated polychromatic erythrocytes (MPE) at 500 mg/kg at 48 h, which was accompanied by mortality and severe bone marrow toxicity. However, in this study, the 24 h time point was not examined. In the second experiment, the number of MPEs was examined in non-iron-supplemented rats at Day 1, 2, 3, 6, and 10 after the treatment at 500 mg/kg. In this study, the number of MPEs was significantly increased ($0.35 \pm 0.11\%$, $0.36 \pm 0.07\%$, $0.44 \pm 0.27\%$, and $0.76 \pm 0.32\%$ at Day 1, 2, 3, and 6, respectively, vs. $0.13 \pm 0.03\%$ in control) at all time points except at Day 10 ($0.15 \pm 0.03\%$). The third experiment was a direct comparison between rats with or without iron-supplemented diet. ICL 670 was found to be negative at 500 mg/kg in both iron-supplemented ($0.20 \pm 0.09\%$, $0.12 \pm 0.04\%$ at 300 and 500 mg/kg, respectively, vs. $0.17 \pm 0.02\%$ in control) and non-iron-supplemented ($0.20 \pm 0.09\%$, $0.23 \pm 0.06\%$ at 300 and 500 mg/kg, respectively, vs. $0.16 \pm 0.05\%$ in control) rats at 48 h time point. In this experiment also, the sponsor did not study the 24 h time point. ICL 670 was found to be positive in non-iron-supplemented rats at 500 mg/kg at 168 h time point ($0.19 \pm 0.12\%$ and $0.58 \pm 0.04\%$ at 300 and 500 mg/kg, respectively, vs. $0.14 \pm 0.04\%$ in control). It is to be mentioned here that similar increase in the number of MPEs was also observed in the previous experiment in non-iron-supplemented rats, where ICL 670 increased the number of MPEs on Day 6 ($0.76 \pm 0.32\%$ vs. $0.13 \pm 0.03\%$ in control). In addition, in a micronucleus assay using hepatocytes from partially hepatectomized rat, ICL 670 was shown to be negative at 250 and 500 mg/kg at 72 and 96 h sampling time.

There was a discussion about the appropriateness of the p53 (+/-) transgenic mouse assay in this case. The p53 (+/-) transgenic mouse assay is primarily useful in evaluation of genotoxicity positive compounds, however, there was an absence of positive genotoxicity findings in iron-supplemented animals, the proposed test design. In addition, the sponsor's dose selection did not appear to be based on any demonstrable end point (doses in excess of those tested in the dose ranging studies were proposed and the body weight effects observed for females did not appear dose-related). The committee had no basis to predict the toxicity of the proposed high dose (600 mg/kg), as this dose has not been tested in iron-supplemented mice. Therefore, the committee was uncertain about the survival of the animal at this dose level for the span of the carcinogenicity study.

Executive CAC Recommendations and Conclusions:

1. The committee did not concur with the sponsor's proposed doses of 30, 100, 300, and 600 mg/kg/day.

2. The appropriateness of the 26-week p53 (+/-) transgenic mouse assay was discussed and was recommended as appropriate for discussion at the PTCC meeting given the absence of positive genotoxicity findings in the iron-supplemented animals.
3. The possibility of the inclusion of non-iron-supplemented animals in the proposed carcinogenicity study was also discussed.

Note: The protocol and the dose selection were discussed by the Pharmacology Toxicology Coordinating Committee (PTCC) on August 16, 2001. The majority considered that the positive genotoxicity results of the micronucleus assays in non-iron supplemented rats are relevant. Therefore, the committee recommended that it is appropriate to conduct p53 (+/-) transgenic mouse assay in both iron supplemented and non-iron supplemented animals, which would help in the interpretation of overall carcinogenic potential of the drug. Inclusion of at least one treatment group without supplementation was recommended.

The committee could not recommend the doses for the non-iron supplemented animals, as there was no data available in non-iron supplemented C57BL/6 mice. However, the committee felt that the doses would likely be lower for the non-iron supplemented mice because of the observed greater toxicity of ICL 670 in other rodents on non-iron supplemented diet.

The PTCC did believe that the doses tested in the male mice in the dose ranging study that yielded decreases in bodyweight gain (200 mg/kg) could be considered an MTD if reproducible, but could not recommend doses for iron-supplemented female mice.

It was felt that kinetics could be collected at study termination using the same animals for tumor analysis, and that the additional satellite treated animals were unnecessary.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD-180
/TChakraborti, HFD-180
/JChoudary, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Joseph DeGeorge
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APPENDIX-4

ExecCAC Meeting Minutes Dated September 17, 2002 for Mouse Carcinogenicity Study

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Executive CAC

September 17, 2002

Committee: David Morse, Ph.D., HFD-150, Acting Chair
Alex Jordan, Ph.D., HFD-580, Alternate Member
Frank Sistare, Ph.D., HFD-910, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the P53 study, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias
This drug is currently under Phase II clinical trial.

P 53 Mouse Carcinogenicity Study Protocol and Dose Selection:

The sponsor has submitted a protocol and dose selection for a 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 20, 100 and 200 mg/kg/day for iron-supplemented animals and 20 mg/kg/day for non-iron-supplemented animals. The sponsor's dose selection was based on the results of the 4-week dose ranging study (study no. 0170121) in C57BL/6 mice with and without iron-supplementation. The sponsor did not submit the actual data or full report of this study. Only summary statements were made.

In the 4-week dose ranging study (study no. 0170121), iron-supplemented C57BL/6 (wild-type) mice were treated with ICL-670 by oral gavage at 100, 300 and 600→450 mg/kg/day. In case of iron-supplemented animals, due to excessive mortality/moribundity

at 600 mg/kg/day following two doses. dosing was suspended on Days 3 – 7 and resumed on Day 8 at 450 mg/kg/day. The animals with standard diet (non-iron-supplemented) were treated at 100 and 600 mg/kg/day.

Iron-Supplemented Diet:

There was no mortality at 100 or 300 mg/kg/day in either sex. However, test article-related mortality was observed in both sexes (three males and one female) at 450 mg/kg/day. The causes of deaths were not discussed. Treatment-related histopathological changes were predominantly observed in males in the following organs at 600→450 mg/kg/day: stomach (gastric erosions and/or ulcers and inflammation in both sexes), cecum (necrosis of epithelial cells in crypts of the cecum), jejunum (hemorrhage), liver (necrosis; however, there was no discussion about the type of necrosis i.e., single cell or focal), adrenal cortex (cytoplasmic vacuolation), lymphoid organs (lymphoid depletion in both sexes, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes), spleen (extramedullary hematopoiesis) and kidney (vacuolation, necrosis and regenerative changes in both sexes and proteinaceous casts). Hematology (increase in hemoglobin in both sexes, hematocrit in males and reticulocyte in males) and serum chemistry (calcium, phosphorus, cholesterol and triglyceride concentrations were increased in males) changes were observed at 300 mg/kg/day. However, the magnitudes of these changes could not be calculated in the absence of any data. In addition, extensive iron-depletion was observed in the liver (83% and 43% reduction in males and females, respectively) and kidney (34% and 46% reduction in males and females, respectively). Based on the mortality and histopathological changes at 600→450 mg/kg/day, the maximum tolerated dose (MTD) in iron-supplemented male mice was considered as 200 mg/kg/day. This MTD estimation is also in agreement with the previous Exec CAC recommendations (August 7, 2001). However, the MTD in females could not be determined in the absence of complete data and full report of this study.

Standard Diet:

There was no mortality in females at 100 mg/kg/day; however, mortality was observed in males at 100 mg/kg/day and at 600 mg/kg/day, all of the animals died or were sacrificed in moribund on Days 3 or 4, following 2 daily doses of ICL-670. The causes of deaths were also not mentioned. Treatment-related histopathological changes were observed in the following organs in both sexes at both doses: stomach (gastric erosions and/or ulcers and inflammation), cecum (necrosis of epithelial cells in crypts of the cecum), jejunum (hemorrhage), liver (necrosis, periportal inflammation containing fibrous tissue and bile duct hyperplasia), lymphoid organs (lymphoid depletion in all animals, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes), spleen (extramedullary hematopoiesis), heart (multifocal myocytolysis) and kidney (vacuolation, necrosis and regenerative changes and proteinaceous casts). The MTD in males appeared to be < 100 mg/kg/day based on mortality and histopathological changes at this dose. The MTD in females appeared to be < 600 mg/kg/day based on mortality at this dose. However, an accurate estimation of MTD in either sex could not be made due to the absence of lower dose levels below 100 mg/kg/day in males and 600 mg/kg/day in females.

Executive CAC Recommendations and Conclusions:

1. The committee concurred with the sponsor's proposed high dose of 200 mg/kg/day for iron-supplemented males. This concurrence was based on the estimation of an MTD from the previous full study (study no. 981060) information and on the results of the current dose ranging study (study no. 0170121) showing mortality at 450 mg/kg/day, which was slightly more than twice the proposed high dose.
2. The Committee did not concur with proposed doses for iron-supplemented females in the absence of a full report of the dose ranging study and complete data. The sponsor should provide full report of the dose ranging study (study no. 0170121) with complete line listing of data.
3. The committee did not concur with the proposed doses for non-iron-supplemented mice due to an inadequate range of doses having been tested in the range finding study. It was recommended that the sponsor should conduct an additional dose ranging study in non-iron-supplemented mice at doses below 100 mg/kg/day in males and between 100 and 600 mg/kg/day in females.

The sponsor should consider conducting histopathological examination in all dose groups. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the control and high dose animals, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group. We note that given the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately or completely described in the recommendations by McConnell et al.

David Morse, Ph.D.
Acting Chair, Executive CAC

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/Division File, HFD-180
/IChoudary, HFD-180
/TChakraborti, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

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this page is the manifestation of the electronic signature.**

/s/

David Morse
9/19/02 05:03:29 PM

APPENDIX-5

ExecCAC Meeting Minutes Dated November 12, 2005 for the Mouse Carcinogenicity
Study

**Appears This Way
On Original**

Executive CAC

November 12, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Josie Yang, Ph.D., HFD-550, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the P53 study, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 58, 554

Drug Name: ICL-670

Sponsor: Novartis Pharmaceuticals Corporation

ICL 670 is an orally active iron chelator intended for the treatment of iron overload in patients with transfusion-dependent anemias
This drug is currently under Phase II clinical trial.

P 53 Mouse Carcinogenicity Study Protocol and Dose Selection:

The sponsor has presented a revised protocol and dose selection for the 26-week oral carcinogenicity study in p53 (+/-) transgenic mice at 20, 100 and 200 mg/kg/day for iron-supplemented males and 30, 100 and 300 mg/kg/day for iron-supplemented females. In this protocol, the sponsor proposed to use 30 and 100 mg/kg/day for males and females on standard diet, respectively. In case of C57BL/6 mice (wild-type), the sponsor proposed to use 200 and 300 mg/kg/day in iron-supplemented males and females, respectively. Sponsor's dose selection was based on the MTD established in the oral 4-week dose ranging study (study no. 0170121) in iron-supplemented C57BL/6 mice at 100, 300 and 600→450 mg/kg/day and in C57BL/6 mice on standard diet at 100 and 600 mg/kg/day. It is to be mentioned here that the sponsor presented the full report of this study in this submission.

In the 4-week dose ranging study (study no. 0170121), iron-supplemented C57BL/6 (wild-type) mice were treated with ICL-670 by oral gavage at 100, 300 and 600→450 mg/kg/day. In case of iron-supplemented animals, due to excessive mortality/moribundity at 600 mg/kg/day following two doses, dosing was suspended on Days 3 – 7 and resumed on Day 8 at 450 mg/kg/day. The animals with standard diet (non-iron-supplemented) were treated at 100 and 600 mg/kg/day.

Iron-Supplemented Diet:

There was no mortality at 100 or 300 mg/kg/day in either sex. However, test article-related mortality was observed in both sexes (three males and one female) at 450 mg/kg/day. The causes of deaths were not discussed. In males, the body weight gain was suppressed by 8%, 18% and 84% at 100, 300 and 600→450 mg/kg/day, respectively, compared to control weight gain. In contrast, the body weight gain in females was increased by 9%, 23% and 64% at 100, 300 and 600→450 mg/kg/day, respectively, when compared to control weight gain. Treatment-related histopathological changes were predominantly observed in males in the following organs at 600→450 mg/kg/day: stomach (gastric erosions and/or ulcers and inflammation in both sexes), cecum (necrosis of epithelial cells in crypts of the cecum in 1 of 10 male), jejunum (hemorrhage in one male), liver (necrosis in 3 of 10 males; necrosis either occurred focally or was seen in scattered hepatocytes throughout liver lobules), adrenal cortex (cytoplasmic vacuolation in 2 of 10 males), lymphoid organs (lymphoid depletion in 6 of 10 males, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 2 of 10 males), spleen (extramedullary hematopoiesis in 2 of 10 animals of each sex), and kidney (tubular necrosis and regenerative changes in the cortex of 8 of 10 males and 1 of 10 females and proteinaceous casts in one male). The MTD in iron-supplemented males appeared to be 200 mg/kg/day based on the mortality and histopathological changes at 600→450 mg/kg/day and severe iron depletion (85%) at ≥ 300 mg/kg/day. However, in case of females, the MTD may be considered as 300 mg/kg/day based on the mortality and histopathological changes (gastric erosions and/or ulcers and inflammation in the glandular stomach, extramedullary hematopoiesis in the spleen, and necrosis and regenerative changes in the kidney) at 600→450 mg/kg/day. Although iron depletion was also observed in the liver and kidney at 300 mg/kg/day in females, however, the extent of this iron depletion is much more severe in males when compared to females (43% iron depletion in the liver in females compared to 83% in males at 300 mg/kg/day).

Standard Diet:

There was no mortality in females at 100 mg/kg/day; however, six males died between Days 13 and 25 and one male was sacrificed in moribund condition on Day 14. At 600 mg/kg/day, all of the animals died or were sacrificed in moribund on Days 3 or 4, following 2 daily doses of ICL-670. The causes of deaths were not mentioned. The body weight gain was suppressed by 92% in males at 100 mg/kg/day compared to control. In contrast, in females, body weight gain at 100 mg/kg/day was similar to control weight gain. In males, serum alanine amino transferase (494% of control, control = 35 U/l), aspartate amino transferase (AST, 184% of control, control = 126 U/l), and alkaline phosphatase (ALP, 382% of control, control = 86 U/l) values were increased at 100

mg/kg/day compared to control. Histopathological changes were observed in the stomach (gastric erosions and/or ulcers and inflammation in males at 100 mg/kg/day and in both sexes at 600 mg/kg/day), cecum (necrosis of epithelial cells in crypts of the cecum in 2 of 10 males and 3 of 10 females at 600 mg/kg/day), liver (necrosis in 8 of 10 males and 2 of 10 females at 100 mg/kg/day, periportal inflammation containing fibrous tissue and bile duct hyperplasia in most males and in one female at 100 mg/kg/day), lymphoid organs (lymphoid depletion in all males at ≥ 100 mg/kg/day and 7 of 10 females at 600 mg/kg/day, infiltration of neutrophils into the cortex of mesenteric and mandibular lymph nodes in 5 of 10 and 6 of 10 males, respectively, and in 2 of 10 and 3 of 10 females at 600 mg/kg/day, respectively), spleen (extramedullary hematopoiesis in 2 of 9 males at 100 mg/kg/day), heart (multifocal myocytolysis in the ventricle in 4 males and 1 female at 600 mg/kg/day) and kidney (vacuolation, necrosis and regenerative changes in 9 of 10 males at 100 mg/kg/day and in 1 of 10 females at 600 mg/kg/day and proteinaceous casts in two males at 100 mg/kg/day). It is to be mentioned here that the histopathological changes at 100 mg/kg/day were predominantly observed in males. The MTD in males appeared to be <100 mg/kg/day based on the mortality and histopathological changes at 100 mg/kg/day. However, in case of females, the MTD may be considered as 100 mg/kg/day based on the mortality at 600 mg/kg/day and absence of any significant histopathological changes at 100 mg/kg/day in females.

Executive CAC Recommendations and Conclusions:

p53 (+/-) Transgenic Mice

1. Iron-Supplemented Diet:

Males: The committee previously concurred with the sponsor's proposed doses of 20, 100 and 200 mg/kg/day for males.

Females:

Based on the MTD derived from observed mortality and histopathological lesions at 450 mg/kg/day, the committee concurred with the sponsor's proposed doses of 30, 100 and 300 mg/kg/day for females.

2. Standard Diet:

Males:

The committee concurred with the proposed dose of 30 mg/kg/day for males based on the observed mortality and histopathology findings at 100 mg/kg/day.

Females:

Based on the MTD derived from observed mortality and histopathological lesions at 600 mg/kg/day, the committee concurred with the sponsor's proposed dose of 100 mg/kg/day for females.

3. C57BL/6 Mice:

Males: The committee concurred with the proposed dose of 200 mg/kg/day for iron-supplemented males.

Females: The committee concurred with the proposed dose of 300 mg/kg/day for iron-supplemented females.

The sponsor should consider conducting histopathological examination in all dose groups. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the control and high dose animals, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group. We note that given the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately or completely described in the recommendations by McConnell et al.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/JChoudary, HFD-180
/TChakraborti, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

Table 3.2
 ICL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented Feed		
		0 mg/kg/day	25 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	70 mg/kg/day	p-cresidine 400 mg/kg/day
RESPIRATORY SYSTEM								
LUNG		(15)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, pleura, focal.								
Score Expanded Totals		1	1	1	2	4	4	7
Inflammation, pleura, diffuse.								
slight				1				
marked				1				
Score Expanded Totals				2				
Inflammation, mixed cell, focal.								
minimal		1				1		
Score Expanded Totals		1				1		2
Leukocytosis.								
minimal							1	
marked			1					
Score Expanded Totals			1				1	
LUNG TUMOR (M).			2					
TRANSITIONAL CELL CARCINOMA (M), metastatic site.								1
Bacteria, pleura.								
severe								
Score Expanded Totals								
TRACHEA		(25)	(25)	(24)	(25)	(25)	(25)	(25)
Within normal limits		23	24	24	24	25	24	25
Inflammation, submucosa, chronic.								
minimal		2						

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study ID: 0230117

5-MAR-04 13:11
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iren				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	20 ng/kg/day	p-cresatidine 400 ng/kg/day
RESPIRATORY SYSTEM								
TRACHEA		(25)	(25)	(24)	(25)	(25)	(25)	(25)
Inflammation, submucosa, chronic.								
slight								
Score Expanded Totals		2					1	1
Inflammation, mixed cell.								
minimal					1			
Score Expanded Totals					1			
LYMPHOMA (M).			1					
SOFT TISSUE								
FAT - MEDIASTINAL				(2)				
Foreign material present, food.								
slight				1				
moderate				1				
Score Expanded Totals				2				
Inflammation, mixed cell.								
slight				1				
marked				1				
Score Expanded Totals				2				
Bacteria.								
slight				1				
moderate				1				
Score Expanded Totals				2				

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 1.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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ORGAN/TISSUE Finding	TREATMENT	IDENTIFICATION OF MICROSCOPIC FINDINGS							
		Males							
		Feed with Iemn				Non-supplemented Feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	20 ng/kg/day	p-aminidine 400 ng/kg/day	
SPECIAL SENSE ORGANS									
EYE		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits				3					2
Atrophy, retina.									
minimal					1				
Score Expanded Totals					1				
Cataract.									
minimal					2				
slight					1				
moderate					4				
marked					1				
Score Expanded Totals					8				
Degeneration, filter, lens.									
minimal		14	12	16	3	8	11	12	
slight		1	3	3	6	2		1	
moderate					2				
marked					1				
Score Expanded Totals		15	15	19	12	10	12	13	
LYMPHOVA (M).									
Mineralization, cornea.									
minimal		1		3					
slight			1						
Score Expanded Totals		1	1	3					
Mineralization, lens.									
slight					1				
Score Expanded Totals					1				
Necrosis, single cell.									
lens.									
minimal							1		
Score Expanded Totals							1		

[M] Malignant Lymphoma
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2
 (M678) 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:11
 Page : 49
 PLACES 2000 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS					
		Males					
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed	
		0 ng/kg/day	20 ng/kg/day	105 ng/kg/day	200 ng/kg/day	0 ng/kg/day	20 ng/kg/day
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Pyknotic, Lens.							
minimal		25	24	22	19	25	23
slight					5		
Score Expanded Totals		25	24	22	24	25	23
HARDERIAN GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		19	14	20	22	14	17
ADENOMA (B)							1
Dilatation, acini.							
minimal		5	7	2	2	4	6
Score Expanded Totals		5	7	2	2	4	6
Hyperplasia, focal.							
minimal						1	
Score Expanded Totals						1	
Infiltrate, lymphocyte.							
minimal		1	5	1	2	1	5
slight		1					1
Score Expanded Totals		2	5	1	2	1	7
Inflammation, mixed cell, focal.							
slight			1				
Score Expanded Totals			1				
Necrosis, focal.							
slight			1				
Score Expanded Totals			1				
LACRIMAL GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits			1	2			1

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

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Table 3.2
 121673: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 12:13
 Page: 56
 PLACES 2000 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed		
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	30 mg/kg/day	p-prosclidine 400 mg/kg/day
SPECIAL SENSE ORGANS								
LACRIMAL GLAND		(25)	(25)	(25)	(25)	(23)	(23)	(24)
Atrophy, acini.								
minimal		10	5	8	15	11	11	7
slight		11	16	14	9	11	10	7
moderate		2				1		
Score Expanded Totals		23	21	22	24	23	21	14
Infiltrate, lymphocytic.								
minimal		14	5	9	14	6	6	9
slight		11	16	14	9	16	13	15
Score Expanded Totals		25	21	23	23	22	23	24
Metaplasia, focal.								
minimal				3	1	3	2	
slight		1					1	
Score Expanded Totals		1		3	1	3	3	
Vascularization, acini.								
minimal					1			
Score Expanded Totals					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-6 (CONTD.)

GROUP 1 to 7 (FEMALES)

Table 3.2.1
 IC6670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 1
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with iron				Non-supplemented feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-600 ng/kg/day	β-cyanoethidine 400 ng/kg/day
CARDIOVASCULAR SYSTEM								
HEART		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		21	24	21	21	21	23	25
Degeneration, myocardium, focal, slight		1						
Score Expanded Totals		1						
Inflammation, epicardium, focal, minimal		1						
slight							1	
moderate		2						
Score Expanded Totals		3					1	
Inflammation, myocardium, focal, minimal								1
slight		1						
Score Expanded Totals		1						1
LYMPHOMA [M]			1					
Vacuolation, myocardium, focal, minimal				1				
slight						1		
Score Expanded Totals				1		1		
Fibrin, epicardium, focal, slight							1	

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 7
 ELACBS 2896 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
CARDIOVASCULAR SYSTEM									
HEART		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Fibrin, epicardium, focal.							1		
Score Expanded Totals									
Inflammation, artery.						1			
slight									
marked		1							
Score Expanded Totals		1				1			
Pyknotic, vascular.									
minimal				1					
Score Expanded Totals				1					
Bacteria, epicardium.									
slight		1			2				
Score Expanded Totals		1			2				
AORTA		(25)	(24)	(25)	(24)	(25)	(24)	(25)	
Within normal limits		25	24	25	24	24	24	25	
Inflammation, artery.									
moderate						1			
Score Expanded Totals						1			
ARTERY		(1)		(1)		(1)			
Polyarteritis.									
slight						1			
moderate		1		1					
Score Expanded Totals		1		1		1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 TCE676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-NR-04 13:24
 Page : 3
 PLACES 2000 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Food with Iron				Non-supplemented food		
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	100-060 mg/kg/day	p-creosidine 400 mg/kg/day
DIGESTIVE SYSTEM								
SALIVARY GLAND								
Within normal limits								
Granules, serous, decreased.		(25) 12	(25) 11	(25) 11	(25) 13	(25) 11	(25) 15	(25) 21
moderate					1			
Score Expanded Totals					1			
Infiltrate, lymphocyte.								
minimal		9	10	10	6	7	8	4
slight		2	4		1	5	2	
Score Expanded Totals		12	14	10	10	12	10	4
Atrophy, parotid, focal.								
slight		1				1	1	1
Score Expanded Totals		1				1	1	1
Mucin, increased.								
slight					1			
Score Expanded Totals					1			
Mucin, decreased, diffuse.								
moderate					1			
Score Expanded Totals					1			
TONGUE								
Within normal limits								
Inflammation, artery.		(25) 24	(25) 25	(25) 25	(25) 25	(25) 24	(25) 25	(25) 25
moderate		1				1		
Score Expanded Totals		1				1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0230117

5-MAR-04 13:24
 Page: 4
 PLACES 2000 V1.002

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-600 mg/kg/day	0-urea/dine 400 mg/kg/day
DIGESTIVE SYSTEM								
ESOPHAGUS								
Within normal limits		(25) 24	(25) 25	(25) 24	(24) 21	(25) 23	(25) 24	(25) 24
Erosion.								
slight		1						
Score Expanded Totals		1						
Fibrosis, muscularis, focal.								
minimal							1	
slight				1	1	2		
Score Expanded Totals				1	1	2	1	
Hemorrhage.								
slight		1						
Score Expanded Totals		1						
Inflammation, adventitia.								
slight					1			
Score Expanded Totals					1			
Bacteria, adventitia.								
minimal					1			
slight					1			
Score Expanded Totals					2			
STOMACH								
Within normal limits		(25) 8	(25) 10	(25) 7	(25) 5	(25) 8	(25) 9	(25) 13
Crystal, limiting ridge.								
minimal						2	2	
slight						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 3370117

5-MAR-04 13:24
 Page : 5
 PLACES 2005 VI.050

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Female					
		Feed with Iron				Non-supplemented feed	
	0 ug/kg/day	30 ug/kg/day	100 ug/kg/day	300 ug/kg/day	0 ug/kg/day	100-500 ug/kg/day	p-cresidine 100 ug/kg/day
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Crystal, limiting ridge.							
Score Expanded Totals						3	2
Cyst, glandular stomach.							
minimal		1					
Score Expanded Totals		1					
Cyst, limiting ridge.							
minimal		5	2	2	4	4	4
Score Expanded Totals		5	2	2	4	4	4
Dilatation, gland, focal.							
minimal		1	11	4	1	1	1
slight				1			
Score Expanded Totals		1	11	5	1	1	1
Edema, non-glandular.							
minimal						1	
slight					1		
Score Expanded Totals					1	1	
Exosion, glandular.							
minimal		1	5	2	5	4	5
slight		1	2	3	1	2	3
moderate					1		
marked					1		
Score Expanded Totals		2	7	5	7	6	8
Hyperkeratosis.							
minimal		1	4	6	13	2	1
slight					1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.1.1
 ICR670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017
 5-MAR-04 13:24
 Page : 6
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Males						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-cresidine 100 ng/kg/day
DIGESTIVE SYSTEM								
STOMACH								
Hyperkeratinosis.								
		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1	4	6	14	2		1
Infiltrate, lymphocyte.								
				1				
Score Expanded Totals		1		1				
Inflammation, glandular, mixed cell, focal.								
minimal		6	7	9	5	6	6	6
slight		1				1	1	1
moderate					1			
Score Expanded Totals		7	7	9	6	7	7	7
Inflammation, submucosa, non-glandular, chronic.								
				5	4	3	4	
minimal		3	1					
Score Expanded Totals		3	1	5	4	3	4	
LYMPHOMA IMI.								
Metaplasia, limiting ridge.								
				1	1	7	1	1
minimal		3	1					
slight				1		1		
Score Expanded Totals		3	1	2	1	8	1	1
Vacuolation, epithelium, glandular.								
					2			
minimal		1			1			
slight								

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 ICL679: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study No. 0270117

5-NRX-04 13:24
 Page : 7
 PLACES 2000 VI.020

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Female					
		Feed with Iron			Non-supplemented feed		
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-300 mg/kg/day	p-cresidine 400 mg/kg/day
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, epithelium, glandular.							
Score Expanded Totals		1			3		
Vacuolation, epithelium, glandular, focal.							
minimal		2	1	1	1		
slight				1			
Score Expanded Totals		2	1	2	1		
Edema, glandular, focal.							
minimal				1			1
Score Expanded Totals				1			1
Hyperplasia, epithelium, glandular, focal.							
minimal						1	
Score Expanded Totals						1	
Hyperplasia, epithelium, non-glandular, focal.							
minimal				1			1
slight						1	
moderate							1
Score Expanded Totals			1		4	2	2
Inflammation, mixed cell, non-glandular, focal.							
minimal					3		
slight						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 FC1670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

3-MAR-04 13:24
 Page : 9
 PLACES 2080 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Fed with Iron			Non-supplemented feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	305 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	0-500 ng/kg/day	
DIGESTIVE SYSTEM									
STOMACH									
Inflammation, mixed cell, non-glandular, focal, moderate		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Score Expanded Totals					3		2	1	
Inflammation, artery, slight				1					
Score Expanded Totals		1		1		1			
Necrosis, glandular, mucosa, focal, minimal								1	
Score Expanded Totals						1			
Ulceration, non-glandular, slight								1	
Score Expanded Totals								1	
Multinucleated giant cell, limiting ridge, minimal									1
Score Expanded Totals								1	
DUODENUM		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	24	24	24	25	24	25	25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.3.1
 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 8-MAR-08 12:24
 Page : 8
 PLACES 2003 01.002

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Food with Iron				Non-supplemented feed	
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-600 mg/kg/day	γ-irradiated feed mg/kg/day
DIGESTIVE SYSTEM							
DUODENUM		(25)	(25)	(25)	(24)	(25)	(25)
Edenation. Brunner's gland.							
minimal						1	
Score Expanded Totals						1	
Hyperplasia. Lymphoid tissue.							
minimal		1	1				
Score Expanded Totals		1	1				
JEJUNUM		(24)	(25)	(25)	(25)	(25)	(25)
Within normal limits		23	23	23	22	22	24
Hyperplasia. Lymphoid tissue.							
minimal				1	1	1	1
slight			2	1	1	1	
moderate				1			
Score Expanded Totals			2	2	2	2	1
Inflammation. Artery.							
slight		1					
Score Expanded Totals		1					
Necrosis. Lymphoid tissue.							
minimal					1		
Score Expanded Totals					1		
Prominent. Smooth cell.							
slight						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1.1
 ICH1676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 5-MAR-04 13:24
 Page : 10
 PLACEI 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
DIGESTIVE SYSTEM									
STOMACH		(24)	(25)	(25)	(25)	(25)	(24)	(25)	
Prominent, Paneth cell.							1		
Score Expanded Totals									
ILEUM		(25)	(25)	(25)	(24)	(25)	(25)	(25)	
Within normal limits		24	23	21	23	26	21	22	
Hyperplasia, lymphoid tissue.									
minimal		1	2	8	1	4	4	3	
slight		1	2	4	1	5	4	3	
Score Expanded Totals									
CECUM		(24)	(25)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		17	20	24	22	24	23	21	
Hyperplasia, crypt.									
minimal			1						
Score Expanded Totals			1						
Hyperplasia, lymphoid tissue.									
minimal		5	1		3		2	2	
slight			3	1		1	1	1	
Score Expanded Totals		5	4	1	3	1	3	3	
Inflammation, mixed cell.									
minimal		1					1		
Score Expanded Totals		1					1		

Figures in parentheses represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q370117
 5-MAR-04 13:24
 Page: 11
 PLATE5 2006 V1.006

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	0-oxcarbazone 400 mg/kg/day
DIGESTIVE SYSTEM							
CECUM		(24)	(25)	(25)	(25)	(25)	(25)
Necrosis, single cell, epithelium, minimal			1				
Score Expanded Totals			1				
Fibrosis, muscularis, slight	1						
Score Expanded Totals	1						
Impaction, moderate	1						
Score Expanded Totals	1						
Inflammation, transmural, minimal	1						
Score Expanded Totals	1						
Necrosis, lymphoid tissue, minimal						1	
Score Expanded Totals						1	
Vacuolation, epithelium, vacuoles contain pyknotic material/inflammatory cells, slight						1	
Score Expanded Totals						1	
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICLE76: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-NR-04 13:24
 Page : 12
 PLACES R000 V1.000

ORGAN/TISSUE Finding		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		TREATMENT	Feed with Iron				Non-supplemented feed	
0 mg/kg/day	30 mg/kg/day		100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	100 mg/kg/day	2,000 mg/kg/day	
DIGESTIVE SYSTEM								
COLON		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.								
minimal		2	2		1			
Score Expanded Totals		2	2		1			
Inflammation, mucosa, focal.								
minimal					1			
Score Expanded Totals					1			
Inflammation, artery.								
slight		1						
Score Expanded Totals		1						
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	23	23	25	23	24	24
Hyperplasia, lymphoid tissue.								
minimal			2	1		2	1	1
slight				1				
Score Expanded Totals			2	2		2	1	1
MESENTERY								
Inflammation, chronic active.								
moderate					1			
Score Expanded Totals					1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page: 13
 PLACES 2886 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Food with Iron				Non-supplemented Feed		
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-160 mg/kg/day	p-creosidine 400 mg/kg/day
DIGESTIVE SYSTEM								
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		3	7	8	13	3	12	11
Atrophy, acinar, focal.			1	1	1			
minimal						1		
marked								
Score Expanded Totals			1	1	1	1		
Basophilia, acinar cell, focal.			1					
minimal			1					1
slight		1						
Score Expanded Totals		1	1			1		1
Dilatation, duct.								
minimal				1				
Score Expanded Totals				1				
Hyperplasia, islet cell.								
minimal		13	12	7	8	12	7	8
Score Expanded Totals		13	12	7	8	12	7	8
Hypertrophy, acinar, focal.								
minimal		2	2	2	2			
Score Expanded Totals		2	2	2	2			
Infiltrate, lymphocyte.								
minimal		9	8	12	8	15	11	7
Score Expanded Totals		9	8	12	8	15	11	7
Inflammation, chronic active, focal.								
slight					1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH698: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 5-MAR-04 13:24
 Page : 14
 PLACES 2000 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	0 mg/kg/day	p-averidine 400 mg/kg/day
DIGESTIVE SYSTEM							
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, chronic active, focal.							
Score Expanded Totals					1	1	
Extragen decreased. minimal		3	3	3	1		
Score Expanded Totals		3	3	3	1		
Decrease, islands of Langerhans. minimal		4	1	1	1	1	2
Score Expanded Totals		4	2	2	1	1	2
Accessory spleen. slight							1
Score Expanded Totals							1
Edema, interstitial. minimal					1		
Score Expanded Totals					1		
Nitotic figures, increased, focal. slight			1				
Score Expanded Totals			1				
Vasculature, acinar cell, focal. minimal		1					
Score Expanded Totals		1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICL690: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study no. CR79117

5-MAR-04 13:24
Page : 15
PLACES 2006 VI.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Food with Iron				Non-supplemented feed		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-cresidine 400 ng/kg/day	
DIGESTIVE SYSTEM								
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Vasopilation, acinar cell, focal.								
Score Expanded Total		2					1	
ACINAR CELL ADENOCARCINOMA (M)								
HEPATOBIILIARY SYSTEM								
GALL BLADDER		(25)	(22)	(24)	(24)	(24)	(25)	(24)
Within normal limits		23	21	20	22	20	21	21
Hyaline nodules, cytoplasm, epithelium								
Slight						1		
Score Expanded Total						1		
Infiltrate, lymphocyte, minimal			1					
Score Expanded Total			1					
Inflammation, lamina propria, mixed cell, minimal		1		2	2	3	4	
Slight				2				1
Score Expanded Total		1		4	2	3	4	1
Inflammation, artery, minimal		1						

(M) Malignant tumor
Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0290117

5-MAR-04 13:24
 Page : 16
 PLACES 2005 V1.068

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Feed				Non-supplemented feed		
		0 mg/kg/day	20 mg/kg/day	100 mg/kg/day	100 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day	P-transidine 400 mg/kg/day
HEPATO-BILIARY SYSTEM								
GALL BLADDER		(25)	(22)	(24)	(24)	(24)	(25)	(24)
Inflammation, artery, Score Expanded Totals		1						
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Within normal limits		2	1	1	3	2	1	
Angiectasia, focal, minimal slight Score Expanded Totals		1		1		1		
Bile stasis, minimal Score Expanded Totals		1		1				5
Focus, eosinophilic, marked Score Expanded Totals				1				1
Glycogen, decreased, hepatocyte, minimal slight Score Expanded Totals					1	1	1	
Hypertrophy, bile duct, minimal slight Score Expanded Totals				1			6	1
				1			6	1
							6	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 DCL676: 26-week oral (gavage) carcinogenicity study in p51 heterozygous mice
 Study no. 0170117

5-MAR-04 13:24
 Page : 17
 PLACES 2000 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Pancreas							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosidine 400 ng/kg/day	
HEPATOBIILIARY SYSTEM									
LIVER									
Hypertrophy, centrilobular.		(25)	(24)	(25)	(25)	(24)	(25)	(25)	
minimal		9	18	13	19	6	11		
slight		1	1	3	2	1	1		
Score Expanded Totals		10	19	16	21	7	12		
Hypertrophy, midzonal.									
minimal									1
slight									20
moderate									4
Score Expanded Totals									25
Inclusion, intranuclear, cytoplasmic invagination.				2					2
minimal									4
slight									13
Score Expanded Totals				2					
Inflammation, lymphocyte, perivascular.									
minimal		7	14	11	11	10	6		2
slight		9		1	2	4			1
Score Expanded Totals		16	14	12	13	14	6		3
Inflammation, mixed cell, focal.									
minimal		4	5	6	2	6	9		2
slight						2	1		
Score Expanded Totals		4	5	6	2	8	10		2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 122678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 5-MAR-04 13:24
 Page: 18
 PLACES 2566 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-300 mg/kg/day	p-terroldine 400 mg/kg/day
HEPATOCHOLELARY SYSTEM							
LIVER		(25)	(24)	(25)	(25)	(24)	(25)
LYMPHOMA (M)			1				
Mitotic figures, increased.				1			2
minimal				1			2
Score Expanded Totals				1			2
Necrosis, centrilobular.							
minimal						1	
Score Expanded Totals						1	
Necrosis, hepatocellular, focal.							
minimal		2	4	3	1	2	3
slight						1	1
Score Expanded Totals		2	4	3	1	3	5
Necrosis, hepatocyte, single cell.							
minimal				1			1
Score Expanded Totals				1			1
Necrosis, midzonal.							
minimal							16
slight							4
Score Expanded Totals							20
Pigment, hepatocellular.							
minimal							1
slight						1	1
Score Expanded Totals						1	1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 FCI670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 5-MAR-04 13:24
 Page : 13
 PLACES 2003 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented Feed			
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-570 400 mg/kg/day	5-creosoline 400 mg/kg/day	
HEPATOBILIARY SYSTEM									
LIVER									
Pigment, Kupffer cell.									
		(25)	(24)	(25)	(24)	(24)	(25)	(25)	
	minimal	1		1			1	18	
	slight			1			1	4	
	Score Expanded Totals	1		2			2	22	
Tension focus.									
	minimal	1	1	1	4	1	5	2	
	slight	1	1	1	1	1	3		
	Score Expanded Totals	2	2	2	5	2	8	2	
Vacuolation, hepatocellular, centrilobular.									
	minimal				1				
	Score Expanded Totals				1				
Vacuolation, hepatocellular, panlobular.									
	minimal		1		1			2	
	slight	1							
	Score Expanded Totals	1	1		1			2	
Vacuolation, hepatocellular, periportal.									
	minimal	13	13	14	5	4	3		
	slight	2		1					
	Score Expanded Totals	15	13	15	5	4	3		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL670: 24-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page: 28
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Irena				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-creosidine 400 ng/kg/day
HEPATOGBILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Extramedullary hematopoiesis.								
minimal		14	15	10	7	11	13	9
slight				2		3	1	7
Score Expanded Totals		14	15	20	7	14	14	16
HEPATOCELLULAR ADENOMA (B).								1
Hyperplasia, nodular, artery.								
slight		1						
Score Expanded Totals		1						
Inflammation, periportal.								
minimal		1		1			6	3
Score Expanded Totals		1		1			6	3
Inflammation, artery.								
minimal		1						
Score Expanded Totals		1						
Inflammation, granulocytic, focal.								
minimal						1		
Score Expanded Totals						1		
Pigment, bile duct.								
minimal							1	
Score Expanded Totals							1	

(B) Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICLETS: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0290117

5-MAR-06 13:34
 Page: 21
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with iron			Non-supplemented feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day
HEPATOBIILIARY SYSTEM								
LIVER		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Prominent, sinusoidal, lining cell.							1	2
minimal				2				
slight				2				
Score Expanded Totals				2			1	2
ENDOCRINE SYSTEM								
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		4	2	4	2	1	5	3
Hypertrophy, cortex, focal.								
minimal		1						
slight		1						
Score Expanded Totals		1						
Hypertrophy, subcapsular cell.								
minimal		20	22	19	21	22	20	21
slight						1		
Score Expanded Totals		20	22	19	21	23	20	21
Hypertrophy, cortex, focal. slight					1	1		
Score Expanded Totals					1	1		
Infiltrate, lymphocyte. minimal						1		
Score Expanded Totals						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL670: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page: 22
 PL/MSB 2000 01.005

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Female					
		Feed with Iron			Non-supplemented feed		
	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-566 mg/kg/day	p. crescens 400 mg/kg/day
ENDOCRINE SYSTEM							
ADRENAL		(24)	(25)	(25)	(25)	(25)	(25)
Necrosis, focal, minimal		1					
Score Expanded Totals		1					
Pigment, medulla, minimal		2	7	2	4	6	6
Score Expanded Totals		2	7	2	4	6	6
Extramedullary Hematopoiesis, minimal		1					
Score Expanded Totals		1		1			
Bacteria, focal, slight		1					
Score Expanded Totals		1					
PARATHYROID		(21)	(21)	(22)	(22)	(22)	(22)
Within normal limits		19	21	19	21	21	21
Hypoplasia, focal, minimal		1					
Score Expanded Totals		1					
Cyst, moderate		1					
Score Expanded Totals		1					
Ectopic thymus, minimal							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICLE70: 28-week oral (gavage) carcinogenicity study in p51 heterozygous mice
 study no. 0270117
 5-MAR-84 13:24
 Days : 28
 PLAUDS 2000 V1.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
	0 ng/kg/day	36 ng/kg/day	108 ng/kg/day	306 ng/kg/day	6 ng/kg/day	108-366 ng/kg/day	0- 408 ng/kg/day	
ENDOCRINE SYSTEM								
PARATHYROID		(21)	(21)	(22)	(22)	(22)	(22)	(22)
Ectopic thymus.				1	1		1	1
slight						1		
moderate								
Score Expanded Totals				1	1	1	1	2
Adema.				1				
minimal				1				
Score Expanded Totals				1				
Infiltrate, lymphocyte.				1				
slight				1				
Score Expanded Totals				1				
PITUITARY		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		24	24	24	24	21	24	25
Cyst, para distalis.								
minimal		1		1		3	1	
Score Expanded Totals		1		1		3	1	
Infiltrate, lymphocyte,								
para distalis, focal.								
minimal						1	1	
Score Expanded Totals						1	1	
LYMPHOMA (M)			1					
THYROID		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Within normal limits		16	21	22	20	22	22	24

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL674: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 8270117

5-NLE-04 13:34
 Page: 24
 FLACS 2005 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Sexes						
		Males				Females		
		Food with Iron				Non-supplemented food		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	p-creosoline 400 ng/kg/day
ENDOCRINE SYSTEM								
THYROID		(25)	(25)	(25)	(24)	(25)	(25)	(25)
Cyst, follicular.								
minimal					1			1
Score Expanded Totals					1			1
Ectopic thymus.								
minimal				1	1	2		
Slight		3	2	1			1	
Score Expanded Totals		3	2	1	1	2	1	
Infiltrate, lymphocyte.								
minimal		4			1		1	
Slight		1				1		
Score Expanded Totals		5			1	1	1	
Inflammation, artery.								
Slight				1				
Score Expanded Totals				1				
Hypertrophy, epithelial.								
minimal		1			1			
Score Expanded Totals		1			1			
Inflammation, focal.								
minimal		1						
Score Expanded Totals		1						
Necrosis, focal.								
minimal		1						
Score Expanded Totals		1						

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICI670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 25
 PLATE# 2800 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-400 ng/kg/day	5-oxoestradiol 400 ng/kg/day
GENITOURINARY SYSTEM								
CERVIX		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25	25
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)
Within normal limits		6	4	11	12	4	2	
Atrophy, tubular, focal.								
minimal								4
slight					1			6
moderate							1	1
marked								1
Score Expanded Totals					1		1	12
Atrophy, tubular, diffuse.								
minimal								1
Score Expanded Totals								1
Eosinophilic tubules.								
minimal		1	7	3	3	6	10	1
slight					1		2	21
moderate					1		1	1
Score Expanded Totals		1	7	3	5	6	12	23
Cast, proteinaceous.								
minimal		13	12	5	5	3	14	3
slight			1		1	3	2	17
Score Expanded Totals		13	13	5	6	11	16	20
Dilatation, tubular.								
minimal								2
slight					1			1

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL679: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0279119

5-MAR-04 13:24
 Page: 26
 CLINCS 2695 V1.009

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Female							
		Feed with Iron			Non-supplemented feed				
		0 mg/kg/day	70 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-600 mg/kg/day	p-terastidine 400 mg/kg/day	
GENITOURINARY SYSTEM									
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)	(25)	
Dilatation, tubular, moderate	Score Expanded Totals				1			1	
Fibrosis, interstitial, focal	Score Expanded Totals				3			3	
minimal								1	
Score Expanded Totals								1	
Hyalinization, glomeruli, minimal				1		1	1		
slight						1			
Score Expanded Totals				1		2	1		
Hydronephrosis, minimal							2		
slight							1		
severe								1	
Score Expanded Totals							3	1	
Hyperplasia, epithelial, pelvis									
minimal						1			
slight								1	
Score Expanded Totals						1		1	
Infiltrate, lymphocyte, minimal		6	7	6	3	9	7	4	
slight		5	1	2		1	4	13	
moderate								1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117
 5-NMF-04 13:24
 Page : 27
 PLACES 2005 V1.096

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
0 ng/kg/day	70 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	p-creosidine 400 ng/kg/day	
GENITOURINARY SYSTEM							
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)
Infiltrate, lymphocyte.							
Score Expanded Totals		11	5	5	3	10	15
LYMPHOMA (M)							
Mineralization, focal.							
minimal		1	2	1	2	2	4
slight							17
Score Expanded Totals		1	2	1	2	2	21
Necrosis, epithelium, tubular, focal.							
minimal							
Score Expanded Totals							
Necrosis, papilla.							
minimal				1			7
slight							5
Score Expanded Totals				1			12
Vacuolation, tubular, epithelium, focal.							
minimal							
slight					1		1
Score Expanded Totals					1		1
Vacuolation, tubular, epithelium, diffuse.							
slight					1		2
Score Expanded Totals					1		2

[N] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 TOL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 28
 ELABOR 2805 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	30 ng/kg/day	300 ng/kg/day	300 ng/kg/day	0 ng/kg/day	300-600 ng/kg/day	p-oxidine 400 ng/kg/day	
GENITOURINARY SYSTEM									
KIDNEY		(15)	(24)	(25)	(25)	(24)	(25)	(25)	
Increase, mesangial cell.				1					
minimal				1					
Score Expanded Totals				1					
Cast, granular.					1				
slight					1				
Score Expanded Totals					1				
Crystal, pelvis.						1			
slight						1			
Score Expanded Totals						1			
Dilatation, collecting tubule.					1		1		
minimal					1		1		
Score Expanded Totals					1		1		
Hyaline droplets, tubular, epithelium.					1				
minimal					1				
slight					1				
Score Expanded Totals					2				
Inflammation, pelvis.					3				
slight					3				
Score Expanded Totals					3				
Inflammation, artery.						1			
moderate						1			
Score Expanded Totals		1				1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 ICLE79: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 8-MAR-04 13:24
 Page : 28
 PLACES 2000 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	5-creosoles 400 ng/kg/day
NON/TOURINARY SYSTEM							
KIDNEY		(25)	(24)	(25)	(25)	(24)	(25)
Bacteria, focal							
minimal		1					
Score Expanded Totals		1					
OVARY		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	18	17	19	15	17
Cyst.							
minimal		1	1			1	
slight				1	2	1	1
moderate					1		
Score Expanded Totals		1	1	1	3	2	1
Dilatation, oviduct.							
minimal						1	
Score Expanded Totals						1	
Hyperthymic, tubal.							
minimal		4	2	6	1	3	2
slight			1		1	1	
Score Expanded Totals		4	3	6	2	4	2
Hyperplasia, lymphoid.							
minimal						4	2
slight		1				1	1
Score Expanded Totals		1				4	3
Hyperplasia, papillary, cystic.							
minimal			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ISL679: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

S-MAR-04 13:24
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 P:\AZEE 2005 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ug/kg/day	30 ug/kg/day	100 ug/kg/day	300 ug/kg/day	0 ug/kg/day	100-500 ug/kg/day	p-oxoestrogen 400 ug/kg/day	
GENITOURINARY SYSTEM									
OVARY									
Hyperplasia, papillary. Cystic.		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Score Expanded Totals			1						
Hyperplasia, tubulostromal.		1	1	1	2	3		2	
minimal					1				
slight		1	1	1	3	1		2	
Score Expanded Totals									
Lymphoma (M).			1						
URINARY BLADDER									
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Acanthosis, epithelium.		15	19	14	13	11	15		
minimal								10	
slight								9	
Score Expanded Totals								19	
Atypia, epithelium, focal.									7
minimal									2
slight									5
Score Expanded Totals									
Hyperplasia, transitional cell, diffuse.									1
minimal									13
slight									11
moderate									

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2000 V1.030

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-cresidine 400 ng/kg/day	
GENITOURINARY SYSTEM									
URINARY BLADDER									
Hyperplasia, transitional cell, diffuse		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
Score Expanded Totals									25
Infiltrate, lymphocyte, mononuclear									
minimal		8	9	10	9	11	7		
slight		2	2				2		
moderate			1		1	1			
Score Expanded Totals		10	12	10	10	12	9		
Inflammation, acute, mixed cell									
minimal									1
Score Expanded Totals									1
Inflammation, mixed cell									
minimal						1			11
slight					1				7
Score Expanded Totals					1	1			18
Inflammation, artery									
minimal		1		1		1			
Score Expanded Totals		1		1		1			
Metaplasia, squamous, epithelium, focal									
minimal									7
slight									14
Score Expanded Totals									21

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 ELACDS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-260 ng/kg/day	p-cresidine 400 ng/kg/day
GENITOURINARY SYSTEM								
PRIMARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Necrosis, epithelium.								14
minimal								1
Slight								15
Score Expanded Totals								5
TRANSITIONAL CELL CARCINOMA (M)								6
TRANSITIONAL CELL PAPILLOMA (B)								
Congestion								
moderate								1
Score Expanded Totals								1
Hemorrhage, submucosa.								1
Slight								1
Score Expanded Totals								1
Hyperplasia, artery.								
minimal				1				
Score Expanded Totals				1				
Mineralization, sarcoma.								
local								
Slight								2
Score Expanded Totals								2
PAPILLOMA (E), squamous cell.								2

(B) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACE# 2003 V1.000

ORGAN/TISSUE/ Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-360 ng/kg/day	p-Enalaprilate 400 ng/kg/day
GEMITOURINARY SYSTEM							
UTERUS		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		5	7	12	8	9	14
Atrophy, endometrium.							
minimal		1			1	1	1
Score Expanded Totals		1			1	1	2
Hyperplasia, cystic endometrial.							
minimal		12	15	13	15	14	9
slight		4	3		1	1	1
Score Expanded Totals		15	18	13	16	15	10
DEVELOPING [M]					1		
(total)					1		
Score Expanded Totals					1		
VAGINA		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		23	24	21	25	21	21
Ectasia, muscularis.							
slight				1			
Score Expanded Totals				1			
Hyperplasia, epithelium.							
minimal		1	1	1		2	4
Score Expanded Totals		1	1	1		2	4
Inflammation, artery.							
moderate						1	

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
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 FLACBS 2000 V1.06B

		INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented feed	
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day
GENITOURINARY SYSTEM							
VAGINA							
Inflammation, artery.		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals						1	
Mucification.						2	
slight		1				2	
Score Expanded Totals		1				2	
HEMOLYMPHATIC SYSTEM							
BONE MARROW							
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)
Granulopoiesis, increased.		11	11	14	18	17	14
minimal		1					
slight		1					1
moderate				2	1	2	
marked		1					
Score Expanded Totals		3		2	1	2	1
EXTRAMED [M]							
Pigment, macrophage.			1				
minimal		8	11	7		3	2
slight		3	2				1
Score Expanded Totals		11	13	7		3	2
Increase, fat.							
minimal			1		1		
Score Expanded Totals			1		1		

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117

5-MAR-04 13:24
 Page : 35
 PLACES 2800 01.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-300 ng/kg/day	3- 400 ng/kg/day	8- 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM								
SCHEMABROW		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Accumulation, macrophage, focal.					1			
slight					1			
Score Expanded Totals					1			
Erythropoiesis, increased.					2	1		
minimal					2	1		
Score Expanded Totals								
Fibrosis, focal.								
minimal	1		1	2	1	1	3	
slight			2				1	
Score Expanded Totals	1		3	2	1	1	4	
Granulopoiesis, decreased.						1		
minimal						1		
Score Expanded Totals						1		
Hypertrophy, lymphoid.					1		1	
minimal					1		1	
Score Expanded Totals					1		1	
Increase, fat.								
minimal					2	1	6	
slight	1		2		1			
Score Expanded Totals	1		2		2	1	6	
New bone formation, focal.								
minimal	1		1	2	2	1	3	
slight	1		2		2			
moderate						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL679: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0290117

5-MAR-04 13:24
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 PLACES 2000 V1.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented Feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-essidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
BONE MARROW								
New bone formation, focal.		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1		1	2	2	2	3
LYMPH NODE - BRONCHIAL								
Within normal limits		(22)	(25)	(23)	(18)	(23)	(24)	(20)
Histiocytosis.		7	7	6	2	8	12	9
minimal		1	2	2		1		
slight					1	1	2	
moderate					1			
Score Expanded Totals		1	2	2	2	2	2	
Infiltrate, mast cell, increased.								
minimal		1					1	1
Score Expanded Totals		1					1	1
Lymphangiectasis.								
minimal		2	6	4		5	2	
Score Expanded Totals		2	6	4		5	2	
LYMPHOMA (M).			1				1	
Pigment, macrophage.								
minimal		8	8	12	3	3	5	16
slight		2	4	2	1	4	2	1
Score Expanded Totals		11	13	14	4	7	7	17
Plasmacytosis.								
minimal		4	5	4	2	5	3	

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 TCELE79: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-KKR-04 13:24
 Page : 17
 PLACES 2980 V1.005

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Doses						
		Feed with Iron			Non-supplemented Feed			
0 ng/kg/day	50 ng/kg/day	100 ng/kg/day	200 ng/kg/day	5 ng/kg/day	100-565 ng/kg/day	p-cresidine 400 ng/kg/day		
HEMOLYTHETIC SYSTEM								
LYMPH NODE - BRONCHIAL		(22)	(25)	(21)	(15)	(23)	(24)	(20)
Plasmacytosis.								
slight		1		1		1		
Score Expanded Totals		5	5	5	2	6	3	
Depletion.								
minimal					2			
slight						1		
Score Expanded Totals					2	1		
Erythroplagocytosis.								
minimal			1					
Score Expanded Totals			1					
Hyperplasia.								
minimal		2			1	2	1	
slight							1	
Score Expanded Totals		2			1	2	2	
Infiltrate, neutrophil.								
minimal				1	1		1	
Score Expanded Totals				1	1		1	
Necrosis.								
minimal					2			
Score Expanded Totals					2			
Plasmacytosis, with Russell bodies.								
slight						1		
Score Expanded Totals						1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH270: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117
 5-MAR-04 13:24
 Page: 28
 PLACES 2000 01.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Female						
		Feed with Iron			Non-supplemented feed			
	5 mg/kg/day	95 mg/kg/day	100 mg/kg/day	300 mg/kg/day	5 mg/kg/day	100-500 mg/kg/day	5-methylthio 400 mg/kg/day	
HEMOLYTHIC SYSTEM								
LYMPH NODE - BRONCHIAL		(22)	(25)	(22)	(18)	(22)	(24)	(25)
Plasmacytosis, Russell bodies.								
minimal								1
Score Expanded Totals								1
Bacteria.								
slight					1			
Score Expanded Totals					1			
LYMPH NODE - BRONCHIAL		(24)	(27)	(25)	(22)	(21)	(24)	(25)
Within normal limits		10	11	12	5	7	9	10
Histiocytosis.								
minimal		5	9	11	10	11	9	12
slight		2				1		
Score Expanded Totals		7	9	11	10	12	9	12
Hypertrophy.								
minimal		1				1	1	
slight								
Score Expanded Totals		1				1	1	
Infiltrate, mast cell, increased.								
minimal		1	3	1	3	2	3	2
Score Expanded Totals		1	3	1	3	2	3	2
Pigment, macrophage.								
minimal		2	1	3	2			1
Score Expanded Totals		2	1	3	2			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 KCL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2396 in.086

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Food with Iron			Non-supplemented feed		
0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	150-300 ng/kg/day	p-irradiated 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MANDIBULAR		(24)	(23)	(25)	(22)	(23)	(25)
Plasmacytosis.							
minimal			1		1	1	2
slight						2	1
Score Expanded Totals			1		1	1	3
Extramedullary							
hematopoiesis.							
minimal				2			1
Score Expanded Totals				2			1
Crystal, macrophage.							
minimal		1					
Score Expanded Totals		1					
Depletion.							
slight						1	
Score Expanded Totals						1	
Hemorrhage.							
slight		1					
moderate							1
Score Expanded Totals		1					1
Infiltrate, neutrophil.							
minimal					1		1
Score Expanded Totals					1		1
Lymphangiectasis.							
minimal		1			1		
Score Expanded Totals		1			1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117

5-MAR-04 13:24
 Page : 40
 PLACER 2000 V1.00B

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Food with ICHN			Non-supplemented feed		
0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosoline 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MANDIBULAR	(24)	(23)	(25)	(22)	(23)	(24)	(25)
Necrosis							
minimal				1			
slight				2		2	
Score Expanded Totals				3		2	
LYMPH NODE - MEDIASTINAL	(1)						
Histiocytosis	1						
Score Expanded Totals	1						
Hyperplasia							
minimal	1						
Score Expanded Totals	1						
LYMPH NODE - MESENTERIC	(25)	(25)	(25)	(25)	(25)	(24)	(25)
Within normal limits	24	22	22	21	23	18	25
Histiocytosis							
minimal	1						
Score Expanded Totals	1						
Infiltrate, neutrophil							
slight				1			
Score Expanded Totals				1			
Inflammation, artery							
moderate			1		1		
Score Expanded Totals			1		1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically.

Table 3.2.1
ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study N5. 0270117

5-MAR-04 13:24
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PLACES 2800 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females				Non-supplemented feed		
		Feed with Iron						
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-crestinine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
LYMPH NODES - MESENTERIC		(25)	(25)	(25)	(25)	(25)	(24)	(25)
Lymphoid hyperplasia.				1				
minimal				1				
Score Expanded Totals				1				
Pigment, macrophage.			3					
minimal			3					
Score Expanded Totals			3					
Aggregation, macrophage.			2					
minimal			2					
Score Expanded Totals			2					
Depletion.					1		3	
minimal					1		3	
slight					1			
Score Expanded Totals					2		3	
Necrosis.			1				4	
minimal			1				4	
Score Expanded Totals			1		1		4	
Necrosis, artery.			1		1		4	
slight								
Score Expanded Totals					1		1	
Extramedullary hematopoiesis.				1				
moderate				1				
Score Expanded Totals				1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0470117
 5-MAR-04 12:24
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 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-uroside 400 ng/kg/day
HEMOLYTHATIC SYSTEM							
LYMPH NODE - PANCREATIC LYMPHOMA (M)							
						(1)	
						1	
THYROID							
Within normal limits							
		(25)	(25)	(26)	(25)	(24)	(25)
Atrophy.							
		18	20	16	12	21	14
							29
					4	1	
		1			2		
					1	1	
							3
		1			7	2	
Score Expanded Totals							
							4
							3
		2	1	5	2		
		1			1		3
		1	1	5	3	2	
Score Expanded Totals							
							3
Hyperplasia, lymphoid.							
		2	3		2		
		1		1			
		1					1
		3	3	1	2		
Score Expanded Totals							
							1
Hyperplasia, epithelial, focal.							
				1			
				1			
Score Expanded Totals							
		1	1				
							2

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL578: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2003 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron				Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	0-400 ng/kg/day	
HEMOLYMPHATIC SYSTEM									
THYRUS		(25)	(25)	(24)	(25)	(24)	(25)	(25)	
Hypersplasia, lymphoid.									
minimal								1	
slight					1		2		
moderate					3		2		
marked							1		
Score Expanded Totals					4		5	1	
Accumulation, macrophage, focal.									
slight				1					
Score Expanded Totals				1					
SPLEEN		(25)	(24)	(25)	(25)	(25)	(25)	(25)	
Within normal limits		10	12	16	11	17	15		
Depletion, lymphoid.									
minimal		1			1	2			
slight		1			2	1	1		
moderate							1		
Score Expanded Totals		2			3	3	2		
Hypersplasia, lymphoid.									
minimal		2		4			1		
slight		1					1		
moderate		1							
Score Expanded Totals		4		4		1	1		
Infiltrate, plasma cell.									
minimal					1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICLEP: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117

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 PLACES 2000 91.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-ercardine 400 ng/kg/day	
HEMOLYMPHATIC SYSTEM									
SPLEEN		(25)	(24)	(25)	(25)	(25)	(25)	(25)	
Infiltrate, plasma cell.									
Score Expanded Totals					1		1		
LYMPHOMA (M)			1					1	
Necrosis, lymphoid.									
minimal					1		2		
moderate					1				
Score Expanded Totals					2		2		
Pigment, macrophage.									
minimal		7	4	3	6	4	1		
slight		3	6	4	2				9
moderate									16
Score Expanded Totals		10	10	7	7	4	1		25
Pigment, melanocyte.									
slight						1			
Score Expanded Totals						1			
Extramedullary hematopoiesis, increased.									
minimal		4	2		4	2	3		
slight		2	1		1		3		15
moderate				1			1		9
marked									
Score Expanded Totals		6	3	2	5	2	4		24
Accessory spleen.			1						

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACEB 2000 01.000

		INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
ORGAN/TISSUE Finding	TREATMENT	Feed with Iron				Non-supplemented feed		
		0 ug/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	p-oxidine 400 ng/kg/day
HEMOLYMPHATIC SYSTEM								
SPLEEN	Congestion.	(25)	(24)	(25)	(25)	(25)	(25)	(25)
	minimal			1				
	slight				1			
	Score Expanded Totals			1	1			
Deformity.	minimal			1				
	slight				1			
	Score Expanded Totals			1	1			
SYSTEMIC/ANY SITE								
	Within normal limits	(25)	(25)	(25)	(25)	(25)	(25)	(25)
	LYMPHOMA (M).	24	24	25	25	25	23	25
	(fatal)	1	1				2	
	Score Expanded Totals	1	1				2	
INTEGUMENTARY SYSTEM								
CLITORAL GLAND								
	Within normal limits	(10)	(14)	(11)	(10)	(11)	(17)	(14)
	Ectasia.	8	10	6	6	7	12	5
	minimal				2			
	slight					1		
	Score Expanded Totals				2	1		
	Hyperplasia, acinar.							
	minimal					1		

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 H2L670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACES 2880 V1.000

ORGAN/TISSUE Finding	TREATMENT	PREVALENCE OF MICROSCOPIC FINDINGS						
		Female						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-600 ng/kg/day	p. crossbred 400 ng/kg/day
INTEGUMENTARY SYSTEM								
CLITORAL GLAND		(19)	(19)	(11)	(10)	(13)	(11)	(14)
Hyperplasia, acinar.								
Score Expanded Totals						1		
Hyperplasia, acinar, diffuse.								
slight			1					
Score Expanded Totals			1					
Inflammation, mixed cell.								
minimal		1	2	3	2	5	1	7
slight			1	2				
Score Expanded Totals		2	3	5	2	5	1	7
MAMMARY GLAND		(24)	(25)	(25)	(25)	(25)	(24)	(25)
Within normal limits		22	25	23	24	21	24	25
Hyperplasia, duct.								
minimal				1	1	2	1	
Score Expanded Totals		2		1	1	2	1	
ADENOCARCINOMA (M), PM1 onset day=121 (fatal)				1				
Score Expanded Totals				1				
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		14	22	23	15	21	21	15

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.1
 ICL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017
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 PLACES 2800 71.089

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	5-eresidine 400 ng/kg/day
IMMUNITARY SYSTEM								
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	
Atrophy, hair follicles.								
minimal			1					
slight				1	1			
Score Expanded Totals			1	1	1			
Fibrosis, dermis, pinna, focal.								
minimal	4	1		2		1	1	
slight				1	1			
moderate		1						
Score Expanded Totals	4	2		3	2	1	2	
Fibrosis, cartilage, pinna, focal.								
minimal	4		1	3	1	1	2	
slight		1	1					
Score Expanded Totals	4	1	2	3	1	1	2	
Hypertrophy, cartilage, pinna, focal.								
minimal							2	
Score Expanded Totals							2	
Inflammation, subcutis, pinna, focal.								
minimal	1		1		1		1	
slight					1		2	
moderate		1			1		2	
Score Expanded Totals	1	1	1	2	1		5	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
Study No. 0270117

5-MAR-04 12:24
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PLACES 2003 V1.096

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	50 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-566 ng/kg/day	p-creatinine 400 ng/kg/day	
INTEGUMENTARY SYSTEM									
SKIN									
Glycer, pinna,		(25)	(25)	(25)	(25)	(25)	(25)	(25)	
minimal		1							
slight								1	
moderate								1	
Score Expanded Totals		1			1			1	
Hyperplasia, epidermis,									
pinna, focal,									
minimal			1						
slight		1							
Score Expanded Totals		1	1						
Inflammation, epidermis,									
focal,									
slight				1					
Score Expanded Totals				1					
Inflammation, subcutis,									
chronic active,									
slight		1							
moderate		1							
marked							1		
Score Expanded Totals		2					1		
Mineralization, cartilage,									
pinna, focal,									
minimal		2							
slight				1					
Score Expanded Totals		2		1				1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 FCL670: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017

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 Page: 48
 PLACES 2808 41.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-60 ng/kg/day	p-cresidine 400 ng/kg/day
IMMUNOGENIC SYSTEM								
SKIN		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Nourish.								
Elight						1		
Score Expanded Totals						1		
Ulcer.								
moderate						1		
Score Expanded Totals						1		
OSTEOSARCOMA [M]. PMI onset day=172.								
(Fatal)		1						
Score Expanded Totals		1						
MUSCULOSKELETAL SYSTEM								
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25	24
OSTEOSARCOMA [M].								1
BONE - STERNUM		(25)	(25)	(25)	(25)	(25)	(25)	(24)
Within normal limits		25	25	25	25	25	24	24
Hypertrophia, cartilage, focal.								
Elight							1	
Score Expanded Totals							1	

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017

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 ELACES 2980 V1.008

ORGAN/TISSUE Finding	TREATMENT	NUMBER OF MICROSCOPIC FINDINGS						
		Males						
		Food with iron				Non-supplemented food		
		0 mg/kg/day	30 mg/kg/day	108 mg/kg/day	308 mg/kg/day	0 mg/kg/day	100-360 mg/kg/day	D-rosiglitazone 400 mg/kg/day
MUSCULOSKELETAL SYSTEM								
MUSCLE								
Within normal limits		(25) 24	(25) 22	(25) 24	(25) 16	(25) 23	(25) 25	(25) 23
Regeneration, myofiber, focal								
minimal					5	1		
Score Expanded Totals					5	1		
Edema								
minimal					1			
Score Expanded Totals					1			
Inflammation, mixed cell, focal					1			
minimal			1					
Score Expanded Totals			1					
Mineralization, myofiber, focal								
minimal					3			
Score Expanded Totals					3			
Regeneration, myofiber, focal								
minimal		1	1	1	5	1		
Slight					1			
Score Expanded Totals		1	1	1	6	1		
Inflammation, mixed cell, abdomen								
Slight								2
moderate								

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLACE 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron			Non-supplemented Feed		
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-560 ng/kg/day	0-CHOLINE 400 ng/kg/day
MUSCULOSKELETAL SYSTEM							
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell, abdominal.							
Score Expanded Totals				1			2
Inflammation, artery, moderate						1	
Score Expanded Totals						1	
Necrosis, artery, slight						1	
Score Expanded Totals						1	
NERVOUS SYSTEM							
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		23	25	23	27	22	25
Dilatation, ventricle, minimal				1	2		
Score Expanded Totals		2		1	2	1	
Inflammation, artery, meninges, slight							
Score Expanded Totals		1		1			
Gitter cell, focal, minimal				1			
Score Expanded Totals		1		1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 FCM570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

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 PLATE 2650 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-oxidine 400 ng/kg/day	
NERVOUS SYSTEM									
BRAIN									
Inflammation, artery, choroid plexus, moderate		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals						1	1		
Necrosis, meningea, artery, moderate				1	1				
Score Expanded Totals									
Necrosis, artery, choroid plexus, slight						1	1		
Score Expanded Totals									
NERVE - SCIATIC									
MICLIN normal limits		(24) 24	(24) 24	(24) 24	(25) 25	(24) 24	(24) 24	(24) 24	(24) 24
SPINAL CORD									
Within normal limits		(25) 25	(25) 25	(25) 25	(25) 25	(25) 25	(25) 25	(25) 25	(25) 25
RESPIRATORY SYSTEM									
LARYNX									
Inflammation, mixed cell, slight						1	1		
Score Expanded Totals						1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 53
 PLACES 2003 V1.000

ORGAN/TISSUE FINDING	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Feed with Iron				Non-supplemented feed	
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-500 mg/kg/day
RESPIRATORY SYSTEM							
LUNG		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		10	15	16	12	8	15
Crystal, focal.				1		1	1
minimal						3	
slight							
moderate							
Score Expanded Totals				1		2	1
Fibrosis, pleural, focal.						4	1
minimal	2	1					1
Score Expanded Totals	2	1					1
Hemorrhage, focal.							
minimal		1			1		1
Score Expanded Totals	1	1			1		1
Histiocytosis, alveolar, focal.							
minimal					1		2
slight				1		2	
moderate							
marked						1	
Score Expanded Totals				1		2	2
Hyperplasia, bronchiolovascular, focal.							
minimal				1		1	1
slight							1
Score Expanded Totals				1		1	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0170117

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 Page : 54
 PLRCSH 2006 v1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Females							
		Feed with Iron			Non-supplemented feed				
		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	6 mg/kg/day	100-550 mg/kg/day	p-cresidine 400 mg/kg/day	
RESPIRATORY SYSTEM									
LUNG		(25)	(25)	(25)	(24)	(25)	(25)	(25)	
Hyperplasia, medial, artery, focal.			1						
minimal			1						
Score Expanded Totals			1						
Hyperplasia, lymphoid tissue.				2	1	4	3	2	
minimal		1	1						
slight		1	1						
moderate		1					1		
Score Expanded Totals		3	2	2	1	5	4	2	
Inflammation, perivascular.				7	6	10	6	5	
minimal		0	0						
slight		1			1	3	2	1	
moderate				1			1		
Score Expanded Totals		1		1	1	3	3	1	
Inflammation, pleura, focal.				2	5	11	5	6	
minimal		1	1				2		
slight		1			3	1			
Score Expanded Totals		2	1		4	1	2		
INFLAMMATION (M)		1	1				1		
Bacteria, pleura, moderate					2				
Score Expanded Totals					2				

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICH670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 55
 PLACES 2680 V1.068

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Sexes					
		Feed with item			Non-supplemented feed		
0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-cresidine 400 ng/kg/day	
RESPIRATORY SYSTEM							
LUNG		(25)	(25)	(25)	(24)	(25)	(25)
Congestion,							
minimal						1	
slight						1	
Score Expanded Totals						2	
Fibrosis, interstitial,							
Total,							
slight						1	
Score Expanded Totals						1	
Hyaline bodies, bronchial,							
epithelium,							
slight						1	
Score Expanded Totals						1	
TRACHEA		(25)	(25)	(25)	(24)	(25)	(25)
Within normal limits		23	23	24	23	21	24
Inflammation, submucosa,							
chronic,							
minimal		1	2	1	1	2	1
Score Expanded Totals		1	2	1	1	2	1
Inflammation, mixed cell,							
minimal		1				2	
Score Expanded Totals		1				2	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.3.1
ICH670: 26-week oral (gavage) carcinogenicity study in p51 heterozygous mice
Study no. 0270117

5-MAR-04 13:24
Page : 56
PLACES 2800 V1.06B

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron			Non-supplemented feed			
		0 ng/kg/day	30 ng/kg/day	300 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-500 ng/kg/day	p-creosidine 300 ng/kg/day
SOFT TISSUE								
FAT			[1]					
Necrosis, abdominal, focal.								
moderate			1					
Score Expanded Totals			1					
FAT - MEDIASTINAL					[3]			
Inflammation, mixed cell.								
slight					1			
moderate					1			
Score Expanded Totals					2			
Bacteria.								
moderate					2			
Score Expanded Totals					2			
SPECIAL SENSE ORGANS								
EYE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		6	8	6	8	6	9	4
Cataract.								
minimal					1			
slight					4		1	
moderate					8			
Score Expanded Totals					13		1	
Degeneration, fiber, lens.								
minimal		5	2	3	1		2	4
slight			2	2	1		1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICLE78: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page : 57
 PLACES 2866 VI.089

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Females					
		Feed with Iron				Non-supplemented feed	
	0 ng/kg/day	30 ng/kg/day	100 ng/kg/day	300 ng/kg/day	0 ng/kg/day	100-600 ng/kg/day	p-creosoline 400 ng/kg/day
SPECIAL SENSE ORGANS							
HTE		(25)	(25)	(25)	(25)	(25)	(25)
Regeneration, fiber, lens.							
moderate					1		
marked					1		
Score Expanded Totals		5	4	5	4	1	0
Mineralization, cornea.							
minimal		1		1		1	
slight							
Score Expanded Totals		1		1		1	
Mineralization, lens.							
slight					1		
Score Expanded Totals					1		
Necrosis, single cell, lens.							
minimal					1		
Score Expanded Totals					1		
Fukusosis, lens.							
minimal		19	16	17	8	15	14
slight			1	2	5		2
moderate					1		
Score Expanded Totals		19	20	19	15	15	16
Inflammation, anterior chamber.							
slight						1	
Score Expanded Totals						1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.1
 ICL676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:24
 Page: 58
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Penicillin						
		Feed with IRRR				Non-supplemented feed		
		0 ng/kg/day	20 ng/kg/day	100 ng/kg/day	200 ng/kg/day	0 ng/kg/day	100-200 ng/kg/day	2-4000 ng/kg/day
SPECIAL SENSE ORGANS								
EYE		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, cornea.								
slight							1	
Score Expanded Totals							1	
Ulceration, cornea.								
slight							1	
Score Expanded Totals							1	
HARDERIAN GLAND		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	18	22	18	19	22	22
Dilatation, acini.								
minimal		1			1			
Score Expanded Totals		1			1			
Infiltrate, lymphocyte.								
minimal		6	6	3	6	6	3	3
slight		1						
Score Expanded Totals		7	6	3	6	6	3	3
Inflammation, mixed cell.								
slight			1					
Score Expanded Totals			1					
LACRIMAL GLAND		(25)	(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		18	16	17	18	8	18	15
Atrophy, acini.								
minimal		5	1		1	2	2	
slight								

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.1
 DCL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 327017

5-MAR-04 13:34
 Page: 53
 PLACES 2836 VI.683

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS						
		Females						
		Feed with Iron				Non-supplemented feed		
		0 mg/kg/day	70 mg/kg/day	100 mg/kg/day	300 mg/kg/day	0 mg/kg/day	100-200 mg/kg/day	2-eresidine 400 mg/kg/day
SPECIAL SENSE ORGANS								
LACRIMAL GLAND		(25)	(22)	(25)	(23)	(25)	(25)	(25)
Atrophy, acini.								
Score Expanded Totals		5	1	1	1	2	2	
Infiltrate, lymphocyte.								
minimal		12	5	7	6	12	5	8
slight		3	1	5	2	7	1	1
Score Expanded Totals		15	10	12	6	14	6	9
Metaplasia, focal.								
minimal		1	1			1		
slight								
Score Expanded Totals		1	1			1		1
Mucin, decreased, diffuse.								
slight					1			
Score Expanded Totals					1			
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES								
HEAD								
Mucosa normal limits					(1) 1			
TAIL								
Mucosa normal limits						(1) 1		(1) 1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-6 (CONTD.)

GROUP 8 AND 9

Table 3.2.2
 ICH676: 26-week oral (gavage) cardiogenicity study in p53 heterozygous mice
 Study no. 0270117r

S-NRR-04 12:28
 Page: 1
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Female
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
CARDIOVASCULAR SYSTEM		
HEART		
Within normal limits	(25) 25	(25) 23
Inflammation, artery, myocardium, marked		1
Score Expanded Totals		1
Inflammation, epicardium, focal, moderate		1
Score Expanded Totals		1
Inflammation, endocardium, focal, moderate		1
Score Expanded Totals		1
AORTA		
Within normal limits	(23) 23	(24) 23
Inflammation, mixed cell, focal, slight		1
Score Expanded Totals		1
ARTERY		
Polyarteritis, moderate		(1) 1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 IC6676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0238117c

5-MAR-04 13:28
 Page: 2
 PLACE 2000 21.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
ESOPHAGUS		
Within normal limits	(25)	(25)
Infiltrate, lymphocytic, minimal	10	13
slight	9	10
Score Expanded Totals	5	2
LYMPHOMA (M)	14	12
	1	
TONGUE		
Within normal limits	(25)	(25)
	25	25
ESOPHAGUS		
Within normal limits	(25)	(25)
Fibrosis, muscularis, focal, minimal	21	29
slight	1	1
Score Expanded Totals	2	3
Inflammation, muscularis, focal, slight	4	4
Score Expanded Totals		1
Degeneration, muscularis, focal, moderate		1
Score Expanded Totals		1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117

5-MAR-04 13:28
 Date : 3
 PLACES 2000 W1.088

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	TREATMENT	
	Non-supplemented Feed	
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
STOMACH	(25)	(25)
Within normal limits	8	11
Cyst, limiting ridge.		
minimal		1
slight	1	2
Score Expanded Totals		3
Dilatation, gland, focal.		
minimal	2	3
Score Expanded Totals	2	6
Erosion, glandular.		
minimal	4	4
slight		1
Score Expanded Totals	4	5
Hypertrophy.		
minimal	2	1
Score Expanded Totals	2	1
Inflammation, glandular, mixed cell, focal.		
minimal	8	10
slight	1	
Score Expanded Totals	9	10
Metaplasia, limiting ridge.		
minimal	2	
slight		1
Score Expanded Totals	2	1
Vacuolation, epithelium, glandular, focal.		
minimal	1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.1.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276119

5-NR-04 13:28
 Page : 4
 PLACES 2000 VI.004

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Parasite
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
STOMACH		
Vacuolation, epithelium, glandular, focal.	(25)	(25)
Score Expanded Totals	1	1
Inflammation, artery.		
marked		1
Score Expanded Totals		1
Inflammation, nonglandular, submucosa, chronic.		
minimal	5	2
slight	1	
Score Expanded Totals	6	2
Cyst, glandular.		
minimal	1	1
Score Expanded Totals	1	1
DUODENUM		
Within normal limits	(25)	(25)
Dilatation, Brunner's gland.	25	24
slight		1
Score Expanded Totals		1
JEJUNUM		
Within normal limits	(25)	(25)
Hyperplasia, lymphoid tissue.	24	25
minimal	1	
Score Expanded Totals	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 K16796: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027011P

5-MAR-04 13:28
 Page : 5
 PLACES 2586 V1.058

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexuals	
	TREATMENT	
	Non-supplemented Feed	
	6 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
ILEUM	(25)	(25)
Within normal limits	23	24
Hyperplasia, lymphoid tissue, minimal	0	1
Score Expanded Totals	23	25
CECUM	(25)	(25)
Within normal limits	21	24
Hyperplasia, lymphoid tissue, minimal	4	1
Score Expanded Totals	25	25
COLON	(25)	(25)
Within normal limits	23	24
Hyperplasia, lymphoid tissue, minimal	2	1
Score Expanded Totals	25	25
RECTUM	(25)	(25)
Within normal limits	24	22
Hyperplasia, lymphoid tissue, minimal	1	3
Score Expanded Totals	25	25
PANCREAS	(25)	(25)
Within normal limits	2	10

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table A.2.2
 K16878: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701171
 5-NOV-04 13:24
 Page : 6
 P1ACRS 2450 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Female
		Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
DIGESTIVE SYSTEM		
PANCREAS		
Atrophy, acinar, focal.		(25)
slight	1	
Score Expanded Totals	1	
Hyperplasia, islet cell.		
minimal	5	9
Score Expanded Totals	5	9
Infiltrate, lymphocyte.		
minimal	14	6
Score Expanded Totals	14	6
Decrease, islets of Langerhans.		
minimal	2	4
Score Expanded Totals	2	4
Pigment, macrophage, focal.		
slight	1	
Score Expanded Totals	1	
Inflammation, focal, chronic.		
slight	1	
Score Expanded Totals	1	
Hemorrhage, focal.		
minimal	1	
Score Expanded Totals	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017E
 5-MAR-04 13:28
 Page : 7
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Animals
		Non-supplemented Food
	6 mg/kg/day	30 mg/kg/day
HEPATOBIILIARY SYSTEM		
GALL BLADDER		
Within normal limits	(25)	(25)
Inflammation, mixed cell, lamina propria.	24	24
minimal	1	1
slight		1
Score Expanded Totals	1	1
LIVER		
Within normal limits	(25)	(25)
Angiectasis, focal.	2	1
minimal		2
Score Expanded Totals		2
Hypertrophy, centrilobular.		
minimal	7	5
Score Expanded Totals	7	5
Hypertrophy, midzonal.		
minimal	1	
Score Expanded Totals	1	
Inflammation, lymphocyte, perivascular.		
minimal	10	3
slight	3	2
Score Expanded Totals	13	11
Inflammation, mixed cell, focal.		
minimal	3	11
Score Expanded Totals	3	11

Figures in () represent the number of animals from which this organ/tissue was examined macroscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0296117

5-MAR-04 13:28
 Page : 8
 PLACES 2606 V1.008

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Animals
		Non-supplemented Feed
	0	33
	mg/kg/day	mg/kg/day
HEPATOBILIARY SYSTEM		
LIVER		
Necrosis, hepatocellular, focal.	(25)	(26)
minimal	4	7
slight	1	
Score Expanded Totals	5	7
Tumors focus.		
minimal	5	1
slight	1	2
Score Expanded Totals	6	3
Vacuolation, hepatocellular, periportal.		
minimal	4	3
slight	1	
Score Expanded Totals	5	3
Extramedullary hematopoiesis.		
minimal	14	16
slight		1
Score Expanded Totals	14	17
Inclusion, intranuclear, cytoplasmic invagination.		
minimal	2	
Score Expanded Totals	2	
Melanin-laden giant cell, focal.		
minimal		1
Score Expanded Totals		1
Inflammation, periportal.		
minimal		1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 NCI570: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701171

5-MAR-04 13:28
 Page : 9
 PLACES 2000 V1.008

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	TREATMENT	
	0	30
	mg/kg/day	mg/kg/day
HEPATOBILIARY SYSTEM		
LIVER		
Glycogen, decreased.	(25)	(25)
slight		1
Score Expanded Totals		1
ENDOCRINE SYSTEM		
ADRENAL		
Within normal limits	(25)	(25)
Hyperplasia, subcapsular cells.	4	5
minimal	20	20
slight	1	
Score Expanded Totals	21	20
Hyperplasia, medulla, focal.		
slight	1	
Score Expanded Totals	1	
PANCREAS		
Within normal limits	(22)	(21)
Cyst.	20	21
minimal	1	
Score Expanded Totals	1	
Ectopic thymus.		
minimal	1	
Score Expanded Totals	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 1.1.2
 126676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0294176

4-MAR-04 13:28
 Page : 18
 PLACES 2003 11.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Sexuals	
		Non-supplemented Food	
		5	30
		mg/kg/day	mg/kg/day
ENDOCRINE SYSTEM			
PITUITARY			
Within normal limits		(25) 25	(25) 25
THYROID			
Within normal limits		(25) 25	(25) 21
Esophageal thyroid.			
minimal		1	2
slight		1	2
Score Expanded Totals		2	2
Infiltrate, lymphocytic.			
minimal			1
Score Expanded Totals			1
Cyst.			
minimal		1	
Score Expanded Totals		1	
Inflammation, artery.			
slight			1
Score Expanded Totals			1
GENITOURINARY SYSTEM			
CERVIX			
Within normal limits		(25) 25	(25) 25
KIDNEY			
Within normal limits		(25) 5	(25) 10

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.2
 K12670: 90-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701177

5-MAR-04 13:28
 Page : 11
 PLACES 2000 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Dosing	
		Non-supplemented Feed	
		0	30
		ng/kg/day	ng/kg/day
GENITOURINARY SYSTEM			
KIDNEY		(25)	(25)
Basophilic tubules.			
minimal			
Score Expanded Totals		4	4
Cast, proteinaceous.		4	4
minimal			
Score Expanded Totals		5	5
Hyalinization, glomeruli.		5	5
minimal			
Score Expanded Totals			1
Infiltrate, lymphocyte.			1
minimal			
slight		10	7
Score Expanded Totals		1	1
Mineralization, focal.		11	3
minimal			
Score Expanded Totals		2	
Vacuolation, tubular, epithelium, focal.		2	
minimal			
Score Expanded Totals			1
Dilatation, pelvis.			1
minimal			
Score Expanded Totals			1
Atrophy, tubular, focal.			1
minimal			
Score Expanded Totals			1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 ICL676: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:28
 Page : 12
 DRACRS 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	
	Non-supplemented Food	
	0 mg/kg/day	30 mg/kg/day
GENITOURINARY SYSTEM		
OVARY	(25)	(25)
Within normal limits	10	14
Cyst.		
minimal	1	1
slight	2	1
Score Expanded Totals	3	2
Hemorrhage, uterine.		
minimal	0	3
slight		2
Score Expanded Totals	0	5
Hyperplasia, luteal.		
minimal	2	1
slight	2	1
Score Expanded Totals	4	2
Hyperplasia, tubular/strat.		
minimal	3	4
slight	1	1
Score Expanded Totals	4	5
UTERINE ENDOMETRIUM	(25)	(25)
Within normal limits	14	7
Infiltrate, lymphocyte, submucosa.		
minimal	11	12
slight		2
Score Expanded Totals	11	14

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.1.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:20
 Page : 13
 PLACES 2000 V1.080

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	TREATMENT	Female
		0 mg/kg/day
GENITOURINARY SYSTEM		
URINARY BLADDER		
Inflammation, artery, moderate		(25) 1
Score Expanded Totals		1
Mineralization, vascular, focal, slight		1 1
Score Expanded Totals		1
UTERUS		
Within normal limits	(25) 15	(25) 11
Atrophy, endometrium, minimal	1	1
slight		1
Score Expanded Totals	1	2
Hyperplasia, cystic endometrial, minimal	7	10
slight	2	2
Score Expanded Totals	9	12
Fibrosis, endometrium, minimal		1
Score Expanded Totals		1
VAGINA		
Within normal limits	(25) 23	(25) 22
Hyperplasia, epithelium, minimal	2	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701172

5-MAR-04 13:28
 Page: 14
 PLACEB 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	FINDINGS	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
GENITOURINARY SYSTEM		
VAGINA		
Hyperplasia, epithelium.	(25)	(25)
Score Expanded Totals	2	2
Inflammation, artery.		1
moderate		1
Score Expanded Totals		1
Metaplasia.		1
slight		1
Score Expanded Totals		1
HEMOLYMPHATIC SYSTEM		
BONE MARROW		
Within normal limits	(25)	(25)
Granulopoiesis, increased.	21	21
minimal	1	
slight	1	
moderate		1
Score Expanded Totals	2	1
Fibrin, focal.		1
minimal	2	
slight	2	1
Score Expanded Totals	2	1
Decrease, fat.		1
minimal	2	1
slight		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICLE70: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02702177

5-MAR-04 13:28
 Page : 15
 PLACES 2850 01.080

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
BONE MARROW	(25)	(25)
Increase, fat.		
Score Expanded Totals	2	2
New bone formation, focal.		
minimal	1	1
slight		1
Score Expanded Totals	2	2
LYMPH NODE - ESPERCHIAL	(22)	(21)
Within normal limits	13	11
Histiocytosis.		
minimal	1	1
Score Expanded Totals	1	1
Infiltrate, mast cell, increased.		
minimal	3	2
Score Expanded Totals	3	2
Lymphangiectasis.		
minimal	2	3
slight		1
Score Expanded Totals	2	3
Pigment, macrophage.		
minimal	2	2
Score Expanded Totals	2	2
Plasmacytosis.		
minimal	1	3
slight		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICLE90: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117E

5-MAR-04 13:28
 Page : 18
 PEACES 2006 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM			
LYMPH NODE - BRONCHIAL		(22)	(21)
Plasmacytosis.			
Score Expanded Totals		1	4
Infiltrate, neutrophil.			
slight			1
Score Expanded Totals			1
LYMPH NODE - MANDIBULAR		(24)	(24)
Within normal limits		8	11
Histiocytosis.			
minimal		10	4
Score Expanded Totals		10	4
Infiltrate, mast cell, increased.			
minimal		4	7
moderate		1	
Score Expanded Totals		5	7
Plasmacytosis.			
minimal		1	1
slight			1
Score Expanded Totals		1	2
Infiltrate, neutrophil.			
minimal		1	
Score Expanded Totals		1	
LYMPH NODE - HEMIPYERIC		(25)	(25)
Within normal limits		24	23

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 ICLEVO: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 3298119c

5-MAR-04 17:28
 Page : 17
 PLACES 2880 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0	30
	mg/kg/day	mg/kg/day
HEMOLYMPHATIC SYSTEM		
LYMPH NODE - MESENTERIC	(25)	(25)
Inflammation, artery.		
mild		1
Score Expanded Totals		1
Necrosis.		
slight		1
Score Expanded Totals		1
LYMPHOMA (N).	1	
THYROID	(25)	(25)
Within normal limits	20	18
Atrophy.		
minimal		1
moderate		1
Score Expanded Totals		2
Cyst.		
minimal	3	5
slight		1
Score Expanded Totals	3	6
Hypertrophy, lymphoid.		
minimal	2	
Score Expanded Totals	2	
Necrosis, lymphoid.		
slight		1
Score Expanded Totals		1

(N) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICH578: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117F

5-MAR-04 13:28
 Page: 18
 PLACES 2000 V1.050

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Panalase	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
HEMOLYMPHATIC SYSTEM		
THYMUS		
Inflammation, artery.	(25)	(25)
slight		1
Score Expanded Totals		1
SPLEEN	(25)	(25)
Within normal limits	24	20
Depletion, lymphoid.		1
moderate		1
Score Expanded Totals		1
Hyperplasia, lymphoid.		2
minimal		2
Score Expanded Totals		2
Pigment, macrophage.	1	
minimal	1	
Score Expanded Totals	1	
Extramedullary hematopoiesis, increased.		1
slight		1
Score Expanded Totals		2
Necrosis, lymphoid.		1
slight		1
Score Expanded Totals		1
SYSTEMIC/ANY SITE	(25)	(25)
Within normal limits	24	25

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 IZL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701177

5-MAR-04 12:28
 Page : 19
 PLACES 2006 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0	30
	mg/kg/day	mg/kg/day
HEMOLYMPHATIC SYSTEM		
SYSTEMIC/ANY SITE	(25)	(25)
Lymphoma (M)	1	
INFEROMENARY SYSTEM		
CLITORAL GLAND	(16)	(13)
Within normal limits	3	3
Ectasia		
minimal	1	2
Score Expanded Totals	1	2
Inflammation, mixed cell		
minimal	7	4
Score Expanded Totals	7	4
MAMMARY GLAND	(25)	(25)
Within normal limits	23	23
Hypertrophy, duct		
minimal	1	1
Score Expanded Totals	1	1
Fibrosis, periductular		
minimal	1	
Score Expanded Totals	1	
Dilatation, duct		
minimal		1
Score Expanded Totals		1

(M) Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 28-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117

5-MAR-04 12:28
 Page : 26
 PLACES 2886 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sexes	
	Non-supplemented Food	
TREATMENT	0 mg/kg/day	30 mg/kg/day
ENTIREMENTARY SYSTEM		
SKIN		
Within normal limits	(25) 16	(25) 17
Fibrosis, dermis, pinna, focal.		
minimal	1	1
slight	4	4
moderate		1
Score Expanded Totals	5	6
Fibrosis, cartilage, pinna, focal.		
minimal	2	1
slight	1	
Score Expanded Totals	3	1
Inflammation, subcutis, pinna, focal.		
minimal	1	
slight	3	2
moderate		3
Score Expanded Totals	4	5
Hypertrophy, epidermis, pinna, focal.		
minimal	2	2
slight	3	1
Score Expanded Totals	5	3
Mineralization, cartilage, pinna, focal.		
slight	1	
Score Expanded Totals	1	
Ulceration, pinna.		
minimal	1	1
slight	2	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 TCM678: 28-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0295117

5-MAR-04 13:28
 Page : 21
 PLACES 2010 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Female	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
INTERUMENTARY SYSTEM		
SKIN	(25)	(25)
Ulceration, pinna. Score Expanded Totals	0	2
Inflammation, subcutis, focal. minimal Score Expanded Totals	1	1
Inflammation, epidermis, pinna, focal. minimal slight Score Expanded Totals	1	1
Necrosis, pinna, focal. minimal Score Expanded Totals	1	1
Hyperplasia, cartilage, pinna, focal. minimal Score Expanded Totals		1
Granuloma. minimal Score Expanded Totals		1
Edema, subcutis, pinna, focal. moderate Score Expanded Totals		1
Cyst, epidermal, inclusion. minimal Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 TCM670: 26-week oral (gavage) carcinogenicity study in p51 heterozygous mice
 Study no. 0270117C

5-MAR-04 13:28
 Page : 22
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		Non-supplemented Feed	
		0 mg/kg/day	30 mg/kg/day
MUSCULOSKELETAL SYSTEM			
BONE - FEMUR/TIBIA		(25)	(25)
Within normal limits		25	25
BONE - STERNUM		(25)	(25)
Within normal limits		25	25
BONE - VERTEBRAE		(1)	
Deformity.			
slight		1	
Score Expanded Tetris		1	
MUSCLE		(25)	(25)
Within normal limits		25	23
Regeneration, myofiber, focal.			1
minimal			1
Score Expanded Tetris			1
Inflammation, artery.			1
moderate			1
Score Expanded Tetris			1
NERVOUS SYSTEM			
BRAIN		(25)	(25)
Within normal limits		24	22
Dilatation, ventricles.			1
minimal		1	1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 MCL678: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0200117E

5-NR-04 13:28
 Page : 23
 PLACES 2000 V1.060

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Female	
		Non-supplemented Feed	
		0	10
		mg/kg/day	mg/kg/day
NERVOUS SYSTEM			
BRAIN		(25)	(25)
Dilatation, ventricle.			1
slight			1
Score Expanded Totals		1	2
Inflammation, artery, meninges.			1
moderate			1
Score Expanded Totals			1
NERVE - SCIATIC		(25)	(25)
Within normal limits		25	22
Regeneration.			1
slight			1
Score Expanded Totals			1
SPINAL CORD		(25)	(25)
Within normal limits		23	24
Nalacia, focal.			1
marked		1	
Score Expanded Totals		1	
Sclerosis, focal.			1
moderate		1	
Score Expanded Totals		1	
Cyst, keratinizing.			1
minimal		1	
Score Expanded Totals		1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL67E: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117E

5-MAR-04 13:28
 Page : 34
 PLACES 2600 VI.003

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS	
		Females	
		0 mg/kg/day	30 mg/kg/day
NERVOUS SYSTEM			
SPINAL CORD			
Inflammation, artery, meninges.		(25)	(25)
slight			1
Score Expanded Totals			1
RESPIRATORY SYSTEM			
LUNG			
Within normal limits		(25)	(25)
Crystal, focal.		10	12
minimal		3	
slight		1	3
Score Expanded Totals		4	3
Histiocytosis, alveolar, focal.			
minimal		2	
slight		2	2
moderate			2
Score Expanded Totals		4	4
Hyperplasia, lymphoid tissue.			
minimal		2	2
slight		1	
Score Expanded Totals		3	2
Inflammation, perivascular.			
minimal		12	7
slight		1	2
Score Expanded Totals		13	9

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 ICH67E: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117c

5-NAR-04 13:28
 Page : 25
 PLACES 2006 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Sex/age	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
RESPIRATORY SYSTEM		
LUNG		
Inflammation, mixed cell, focal.	(25)	(25)
minimal	1	2
Score Expanded Totals	1	2
Hypertrophy, artery, medial, focal.		
minimal	1	1
slight	1	1
Score Expanded Totals	2	2
Hypertrophy, pneumocyte Type II, focal.		
minimal	1	1
slight	1	2
Score Expanded Totals	2	3
Granuloma.		
minimal	1	
Score Expanded Totals	1	
Bacteria, pleura.		
slight		1
Score Expanded Totals		1
Necrosis, focal.		
moderate		1
Score Expanded Totals		1
Inflammation, pleura.		
marked		1
Score Expanded Totals		1
Fibrosis, focal.		
minimal		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0275177

5-MAR-04 13:28
 Page: 26
 PLACES 2680 VI.039

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0	30
	mg/kg/day	mg/kg/day
RESPIRATORY SYSTEM		
LUNG		
Fibrosis, focal.	(25)	(25)
Score Expanded Totals		1
Fibrin, vascular.		1
slight		1
Score Expanded Totals		1
Fibrin, focal.		1
minimal		1
Score Expanded Totals		1
TRACHEA		
Within normal limits	(25)	(25)
Inflammation, submucosa, chronic.	25	22
minimal		3
Score Expanded Totals		3
SPECIAL SENSE ORGANS		
EYE		
Within normal limits	(25)	(25)
Degeneration, fiber, lens.	4	3
minimal		
Score Expanded Totals	5	6
Pyknotic, lens.	5	6
minimal	20	22
slight	1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICL670: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02701171

5-MAR-04 13:28
 Page : 27
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Food
	0 mg/kg/day	30 mg/kg/day
SPECIAL SENSE ORGANS		
EYE	(25)	(25)
Pyknosis, lens.		
Score Expanded Totals	21	22
HARDERIAN GLAND	(25)	(25)
Within normal limits	19	20
Dilatation, acini.		
minimal	3	3
Score Expanded Totals	2	3
Infiltrate, lymphocyte.		
minimal	4	3
Score Expanded Totals	4	3
LACRIMAL GLAND	(21)	(21)
Within normal limits	8	11
Atrophy, acini.		
minimal	2	3
slight	7	1
Score Expanded Totals	5	4
Infiltrate, lymphocyte.		
minimal	7	8
slight	5	2
Score Expanded Totals	12	10
Metaplasia, focal.		
minimal	1	
slight		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICLE70: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:28
 Page : 28
 PLACES 2890 VI.058

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS	
	Females	
	TREATMENT	Non-supplemented Feed
	0 mg/kg/day	30 mg/kg/day
SPECIAL SENSE ORGANS		
LACRIMAL GLAND		
Metaplasia, focal	(22)	(23)
Score Expanded Totals	1	1
Necrosis, duct		
minimal	1	
Score Expanded Totals	1	
TOPOGRAPHIC REGIONS/CELLULAR STRUCTURES		
MEDIASTINUM		
Inflammation, chronic active, around esophagus with bacteria		(1)
marked		1
Score Expanded Totals		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

APPENDIX-6 (CONTD.)

WILD TYPE MICE

Table 3.2.3
 ICL607: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117

5-MAR-04 13:35
 Page : 1
 PLACES 2000 V1.000

GROSS/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
CARDIOVASCULAR SYSTEM							
HEART		(25)	(25)	(25)	(25)	(25)	(25)
Vasculature, myocardium, focal.							
minimal		2	4	1			
Score Expanded Totals		2	4	1			
Proliferation, myzoid, mitral valve.							
minimal			2	1			
Score Expanded Totals			2	1			
Mineralization, myocardium, focal.							
minimal					2		
Score Expanded Totals					2		
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Inflammation, myocardium, focal.							
minimal		1					
Score Expanded Totals		1					
Inflammation, epicardium.							
minimal					1		
Score Expanded Totals					1		
Atrophy, myocardium.							
minimal				1			
Score Expanded Totals				1			
Within normal limits		22	20	20	22	25	24
AORTA		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	24	25	25	25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.1.3
 ICL667:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0298117w
 5-NR-04 12:35
 Page : 2
 PLACEB 2602 VI.00F

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		0 mg/kg/day	6 mg/kg/day	200 mg/kg/day	0 mg/kg/day	6 mg/kg/day	300 mg/kg/day
CARDIOVASCULAR SYSTEM							
ARTERY							
Dysenteritis, moderate							(1)
Score Expanded Totals							1
DIGESTIVE SYSTEM							
PANCREAS							
Mineralization, duct, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1	1	1	1	1	1
INTESTINE							
Metaplasia, squamous, duct, focal, minimal		1					
Score Expanded Totals		1					
Infiltrate, lymphocytic, minimal		14	12	13	13	13	14
Score Expanded Totals		4	4	6	2	3	4
Hypoplasia, ductular, focal, minimal		19	16	19	18	14	18
Score Expanded Totals		1					
STOMACH							
Dilatation, duct, minimal			1				
Score Expanded Totals			1				
ESOPHAGUS							
Atrophy, acini, focal, minimal							1
Score Expanded Totals							1
Within normal limits		7	8	6	10	11	6

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.3
 MULEST: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 6279117w

S-MNR-04 13:28
 Page : 3
 PLACDS 2008 V1.090

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day
DIGESTIVE SYSTEM							
ESOPHAGUS		(25)	(25)	(25)	(25)	(25)	(25)
MALT CELL TUMOR [B].				1			
Inflammation, atrophy.							
slight							1
Score Expanded Totals							1
Inflammation, focal, chronic.							
minimal		1					
Score Expanded Totals		1					
Within normal limits		24	25	24	25	25	24
ESOPHAGUS		(25)	(25)	(25)	(25)	(25)	(25)
Regeneration, myofiber, muscularis.							
minimal							1
Score Expanded Totals							1
Hyperkeratinosis.							
minimal			1				
slight			1				
Score Expanded Totals			2				
Within normal limits		25	23	25	25	25	24
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Ulceration, nonglandular.							
minimal							1
moderate							
Score Expanded Totals							1
Vascularization, epithelium, glandular, focal.							
minimal			2	2		1	1

[B] Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL697: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0279117W
 5-MAR-04 13:35
 Page 4
 PLACE# 2005 WJ.004

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males				Females	
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day		
DIGESTIVE SYSTEM							
STOMACH		(25)	(25)	(25)	(25)	(25)	(25)
Vasculature, epithelium, glandular, focal.							
Score Expanded Totals			2	2		1	1
Metaplasia, limiting ridge, minimal		1	2	1	1		2
Score Expanded Totals		1	2	1	2		2
Inflammation, artery, moderate							1
Score Expanded Totals							1
Inflammation, mixed cell, non-glandular, focal, moderate							1
Score Expanded Totals							1
Inflammation, submucosa, non-glandular, chronic, minimal		2	1	2	5	8	4
Score Expanded Totals		2	1	3	5	8	5
Inflammation, glandular, mixed cell, focal, minimal		4	6	10	8	3	4
Score Expanded Totals		4	7	11	6	7	5
Infiltrate, lymphocyte, minimal							1
Score Expanded Totals							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-NAK-04 12:15
 Page: 5
 PLACES 2010 V1.000

ORGAN/TISSUE Finding	INCIDENCE OF MICROSCOPIC FINDINGS						
	TREATMENT	Males				Females	
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
DIGESTIVE SYSTEM							
STOMACH							
Hyperplasia, glandular, focal, minimal	(25)	(25)	(25)	(25)	(25)	(25)	
slight				1	1		
moderate		1					
Score Expanded Totals		1		1	1		
Hyperplasia, epithelium, nonglandular, focal, minimal							3
slight							1
Score Expanded Totals							4
Hyperkeratosis, minimal				3	1		14
Score Expanded Totals				3	1		14
Foreign material present, hair, nonglandular, minimal							1
Score Expanded Totals							1
Erosion, glandular, minimal	4	2	2	3	3		6
slight	3		1	1			2
Score Expanded Totals	5	2	3	3	3		6
Edema, submucosa, nonglandular, slight			1				
Score Expanded Totals			1				
Edema, glandular, minimal							1
Score Expanded Totals							1
Dilatation, gland, focal, minimal		2	3	4	1		2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICH587:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no: 0270117w

5-MAR-04 13:35
 Page : 6
 PLACEB 2000 Y1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
	D mg/kg/day	S mg/kg/day	200 mg/kg/day	0 mg/kg/day	5 mg/kg/day	300 mg/kg/day	
DIGESTIVE SYSTEM							
STOMACH							
Dilatation, gland, focal, slight		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals			2	1	4	1	2
Cyst, limiting ridge, minimal		2	3	2	3	2	4
Score Expanded Totals		2	3	2	3	2	4
Cyst, glandular, minimal			2		1		
Score Expanded Totals			2		1		
Within normal limits		15	13	11	6	12	3
DUODENUM							
Hyperplasia, lymphoid tissue, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals							1
Within normal limits		25	25	25	25	25	24
JEJUNUM							
Hyperplasia, lymphoid tissue, slight		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		1	1				
Within normal limits		24	24	25	25	25	25
ILEUM							
Inflammation, mixed cell, focal, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals				1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICH807.26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
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 PLACES 2640 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
DIGESTIVE SYSTEM							
ILEUM		(25)	(25)	(25)	(25)	(25)	(25)
Hypertrophy, lymphoid tissue.							
minimal		2	2		1		
Score Expanded Totals		2	2		1		
Within normal limits		23	23	24	24	25	25
CECUM		(25)	(25)	(25)	(25)	(25)	(25)
Necrosis, single cell, epithelium.							
minimal					1		
Score Expanded Totals					1		
Inflammation, mixed cell, focal.							
slight					1		
Score Expanded Totals					1		
Infiltrate, lymphocyte.							
minimal			1				
Score Expanded Totals			1				
Hypertrophy, lymphoid tissue.							
slight		1					
Score Expanded Totals		1					
Edema, submucosa.							
minimal			2				
Score Expanded Totals			2				
Within normal limits		24	23	23	25	25	25
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Lymphangiectasis.							
minimal			1				
Score Expanded Totals			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 ICH607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-NDR-04 13:15
 Page : 6
 PLACES 2806 VI.005

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	8 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
DIGESTIVE SYSTEM							
COLON		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		1	2				1
Score Expanded Totals		1	2				1
Mucosa, submucosa.							
minimal			1				
Score Expanded Totals			1				
Within normal limits		24	22	25	25	25	24
RECTUM		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal					2	3	2
slight							1
Score Expanded Totals					2	3	3
Within normal limits		25	25	25	23	24	22
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)
Decrease, islets of Langerhans.							
minimal					2	4	1
slight			1				
Score Expanded Totals			1		2	4	1
Synogen decreased.							
minimal			4	1		1	
Score Expanded Totals			4	1		1	
Inflammation, mixed cell, focal.							
minimal							1
Score Expanded Totals							1
Infiltrate, lymphocyte.							
minimal		9	22	19	15	19	12
slight		1		1	1		

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.3
 ICL687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

9-MAR-04 13:35
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 M3ACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
DIGESTIVE SYSTEM							
PANCREAS		(25)	(25)	(25)	(25)	(25)	(25)
Infiltrate, lymphocyte.							
Score Expanded Totals		10	12	14	16	12	12
Hypertrophy, vacuolar, focal.							
slight					1	1	
moderate		1					
Score Expanded Totals		1			1	1	
Hypertrophy, acinar, focal.							
minimal		2		2			1
slight							1
Score Expanded Totals		2		2			2
Hypertrophy, islet cell.							
minimal		10	12	14	15	11	12
slight		1	1				
Score Expanded Totals		11	13	14	15	11	12
Eosinophilia, acinar cell, focal.							
minimal		1	2	1			
Score Expanded Totals		1	2	1			
Atrophy, acinar, focal.							
minimal			1		2	1	1
slight							1
Score Expanded Totals			1		2	1	2
Within normal limits		9	4	5	3	5	9

Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:35
 Page: 10
 PLACES 2496 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEPATOBIILIARY SYSTEM							
GALL BLADDER		(25)	(23)	(24)	(25)	(25)	(25)
Inflammation, mixed cell, lamina propria.							
minimal		2	5	3	4	5	5
slight		1		1		1	1
Score Expanded Totals		3	5	4	4	7	6
Edema, lamina propria.							
minimal		1		1			
slight				1			
Score Expanded Totals		1		2			
Within normal limits		21	18	19	21	18	19
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Extracellular hematopoiesis.							
minimal		11	15	14	15	12	14
slight		1			1	2	
Score Expanded Totals		12	15	14	16	14	14
Vacuolation, hepatocellular, periportal.							
minimal		4	3		15	5	10
slight		2			0		
Score Expanded Totals		6	3		15	5	10
Vacuolation, hepatocellular, periportal.							
minimal		1	4	2			
slight				1			
moderate		1					
marked		2					
Score Expanded Totals		4	4	3			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL697: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q270117a
 5-MAR-04 13:35
 Page: 11
 PLACES 2885 V1.088

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 ng/kg/day	0 ng/kg/day	200 ng/kg/day	0 ng/kg/day	0 ng/kg/day	300 ng/kg/day
HEPATOBIILIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, hepatocellular, centrilobular.							
minimal			6	0			
slight		4	0	7			
moderate		9	2	5			
marked		2	2	1			
Score Expanded Totals		15	14	15			
Vacuolation, hepatocellular.							
minimal			1				
Score Expanded Totals			1				
Tension focus.							
minimal			1	2		1	1
slight			2			1	
Score Expanded Totals			3	2		2	1
Necrosis, hepatocellular, focal.							
minimal		3	9	2	6	12	7
slight						3	1
moderate						1	
Score Expanded Totals		3	9	2	7	15	8
Karyomegaly, hepatocellular, centrilobular.							
minimal		1	1	3			
Score Expanded Totals		1	1	3			
Inflammation, artery.							
moderate		1	1	1			
Score Expanded Totals							1
Inflammation, mixed cell, focal.							
minimal							1
slight		2	9	6	11	15	10
Score Expanded Totals						2	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL607: 26-week oral (gavage) cardiotoxicity study in p53 heterozygous mice
 Study no. 0270117w

5-NR-04 17:15
 Page : 12
 PLACEI 2890 V1.06B

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
0 mg/kg/day	5 mg/kg/day	200 mg/kg/day	0 mg/kg/day	5 mg/kg/day	200 mg/kg/day		
HEPATOBILLIARY SYSTEM							
LIVER		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell, focal.							
Score Expanded Totals		2	5	6	11	17	10
Inflammation, periportal.							
minimal							
Score Expanded Totals						1	1
Inflammation, lymphocyte, perivascular.						1	1
minimal		4	6	8	4	8	3
slight		2	5	2	1	2	
Score Expanded Totals		6	11	10	5	10	3
Inclusion, intranuclear, hyaline.							
minimal				1			
Score Expanded Totals				1			
Inclusion, intranuclear, invaginated cytoplasm.							
minimal				1			
Score Expanded Totals				1			
Inclusion, intranuclear, cytoplasmic invagination.							
minimal		4	1	2			
Score Expanded Totals		4	1	2			
Inclusion, intranuclear, cytoplasmic invagination.							
minimal				1			
Score Expanded Totals				1			
Hypertrophy, hepatocellular, centrilobular.							
minimal		22	22	22	7	2	5
slight		2		2			2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 122637:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q590117w

5-MAR-04 13:35
 Page : 13
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 ng/kg/day	0 ng/kg/day	200 ng/kg/day	0 ng/kg/day	0 ng/kg/day	300 ng/kg/day
HEPATOCELLULAR SYSTEM							
LIVER							
Hypertrophy, hepatocellular, centrilobular.		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals		24	22	25	7	2	10
Hyperplasia, nodular, artery, moderate							1
Score Expanded Totals							1
HISTIOCYTIC SARCOMA (H)					1		
Focus, sinus cell, slight			1				
Score Expanded Totals			1				
Dilatation, vascular, minimal			1				
Score Expanded Totals			1				
Kidney normal limits						3	4
ENDOCRINE SYSTEM							
ADRENAL							
Pigment, medulla, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals					3	6	3
Hypertrophy, cortex, focal, minimal		2		1			
Score Expanded Totals		2	1	1			
Hyperplasia, subcapsular cell, minimal		5	2	6	22	22	23

(H) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.8.3
 IELC97: 96-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276179

5-NBR-04 13:35
 Page: 14
 PHARM 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 200 ng/kg/day	Feed w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Feed w/ Iron 300 ng/kg/day
ENDOCRINE SYSTEM							
ADRENAL		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, subcapsular cell. Score Expanded Totals		5	5	5	22	22	23
Hyperplasia, medulla, diffuse. slight Score Expanded Totals			1				
Mitotic, vein. minimal Score Expanded Totals			1	1			
Atrophy, cortex. minimal Score Expanded Totals			1	1			
Within normal limits		19	18	17	2	2	2
PARATHYROID		(22)	(23)	(23)	(23)	(24)	(25)
Hyperplasia, fucal. minimal Score Expanded Totals							2
Ectopic thymus. minimal slight Score Expanded Totals						1	1
Cyst. minimal slight Score Expanded Totals				1	1	2	1
Within normal limits		22	23	22	21	21	21

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 102407:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027017a

5-MAR-04 13:35
 Page : 15
 PLACES 2000 V1.005

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
	0	5	25	0	0	100	
		mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
ENDOCRINE SYSTEM							
PITUITARY							
Cyst, para distalis.		(25)	(25)	(25)	(25)	(25)	(24)
minimal		5	2	3	2		1
slight			2				
Score Expanded Totals		5	4	3	2		1
Within normal limits		20	21	22	24	25	23
THYROID							
Inflammation, artery.		(25)	(25)	(25)	(25)	(25)	(25)
moderate							1
Score Expanded Totals							1
Infiltrate, lymphocyte.							
minimal		2				1	1
Score Expanded Totals		2				1	1
Ectopic thymus.							
minimal				1	1		
slight		1				1	
Score Expanded Totals		1		2	1	1	
Cyst, follicular.							
minimal		1					1
slight					1		
Score Expanded Totals		1			1		1
Within normal limits		21	25	23	23	23	22
GENITOURINARY SYSTEM							
CERVIX							
Within normal limits					(25) 25	(25) 25	(25) 25

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 JCL667:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117w

5-MAR-04 13:35
 Page: 16
 PLACES 2680 01.080

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males				Females	
		Feed w/ 0 mg/kg/day	No 0 mg/kg/day	Feed w/ 200 mg/kg/day	Feed w/ 0 mg/kg/day	Feed w/ 0 mg/kg/day	Feed w/ 300 mg/kg/day
GENITOURINARY SYSTEM							
EPIDIDYMIDES							
Vacuolation, epithelium, increased, focal.		(25)	(25)	(25)			
minimal		1	2				
Score Expanded Totals		1	2				
Oligospermia.							
slight		2					
moderate			1				
marked		1		1			
Score Expanded Totals		3	1	1			
Lymphangiectasis.							
minimal		2	5	1			
Score Expanded Totals		2	5	1			
Inflammation, interstitial, chronic.							
minimal		1					
Score Expanded Totals		1					
Infiltrate, lymphocyte.							
minimal		3	12	7			
slight		4	1	3			
Score Expanded Totals		7	13	10			
Hyperplasia, epithelium, focal.							
minimal		2					
Score Expanded Totals		2					
Granuloma, spermatic.							
slight		1	1				
moderate		1	1				
marked				1			
Score Expanded Totals		2	2	1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL657: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117

5-MAR-04 13:15
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 PLACES 2000 VI.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 5 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 250 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMATOPOIETIC SYSTEM							
SPLEEN							
Fibrosis, interstitium.		(25)	(25)	(25)			
minimal		1		1			
slight		2	1	1			
Score Expanded Totals		3	1	2			
Edema, interstitium.							
minimal				1			
slight			1	1			
Score Expanded Totals			1	1			
Degeneration, epithelium, focal.							
minimal			1				
Score Expanded Totals			1				
Debris, increased, lumen.							
minimal		1	1				
slight		1					
Score Expanded Totals		2	1				
Cleft, cholesterol.							
slight			1				
Score Expanded Totals			1				
Within normal limits		21	9	13			
LIVER							
Increase, hexagonal cell.		(25)	(25)	(25)	(25)	(25)	(25)
minimal		1		2			
Score Expanded Totals		1		2			
Vacuolation, tubular, epithelium.							
diffuse.							
marked				1			
Score Expanded Totals				1			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 8278117w

5-MAR-04 13:35
 Page : 18
 PLACES 2000 V1.089

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, tubular, epithelium, focal.							
minimal		14	18			1	1
slight		6	4				1
Score Expanded Totals		20	22			1	2
Dyskinesis, nasangial cell.							
minimal			1	1			
Score Expanded Totals			1	1			
Mineralization, focal.							
minimal		7	2	2	2	1	1
Score Expanded Totals		7	2	2	2	1	1
Infiltrate, lymphocyte.							
minimal		6	12	13	10	7	7
slight			1	1	4	5	3
Score Expanded Totals		6	13	14	14	12	10
Hyalinization, glomerulus.							
minimal		1	1	1	2		
Score Expanded Totals		1	1	1	2		
Hyaline droplets, tubular, epithelium.							
moderate					1		
Score Expanded Totals					1		
Fibrosis, focal.							
minimal					1		
Score Expanded Totals					1		
Ectopic tissue, bone.							
minimal					2		1
Score Expanded Totals					2		1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 IC5687: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117a

5-MAR-04 12:35
 Page : 19
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
GENITOURINARY SYSTEM							
KIDNEY		(25)	(25)	(25)	(25)	(25)	(25)
Deposition, fat, glomerular.							
minimal		1	4	1			
Score Expanded Totals		1	4	1			
Cast, proteinaceous.							
minimal		8	5	8	10	11	6
Score Expanded Totals		8	5	8	10	11	6
Basophilic tubules.							
minimal		7	11	5	5	5	8
Slight					1		
Score Expanded Totals		7	11	5	6	5	8
Within normal limits		1		5	6	5	6
OVARY					(25)	(25)	(25)
Hyperplasia, tubulocystic.							
minimal					2	1	1
Slight						1	1
Score Expanded Totals					2	2	2
Hyperplasia, lymphoid.							
minimal					1	1	1
Slight					1		
Score Expanded Totals					2	1	1
Hyperplasia, cystic, papillary.							
minimal						1	
Score Expanded Totals						1	
Hemorrhage, tubal.							
minimal					12	5	4
Slight						2	
Score Expanded Totals					12	7	4

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.2
 IRL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-MAR-04 12:35
 Page: 28
 PLACE3 2000 V1.063

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	Feed w/ Iron + Supplement 0 mg/kg/day	Feed w/ Iron + Supplement 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	Feed w/ Iron + Supplement 0 mg/kg/day	Feed w/ Iron + Supplement 300 mg/kg/day
GENITOURINARY SYSTEM							
OVARY					(25)	(25)	(25)
Dilatation, evident.							
minimal							1
Score Expanded Totals							1
Cyst.							
minimal							1
slight					1	2	2
Score Expanded Totals					1	2	3
Within normal limits					11	11	16
PROSTATE		(24)	(25)	(25)			
Contant, impregnated, focal.							
minimal		2	3	2			
slight		2	2	1			
moderate		1					
Score Expanded Totals		5	5	3			
Vacuolation, epithelium, focal.							
minimal			1				
Score Expanded Totals			1				
Lymphangiectasis.							
slight			1				
Score Expanded Totals			1				
Infiltrate, lymphocyte, ganglion.							
minimal		3	1				
slight			1				
moderate		1		1			
marked		1					
Score Expanded Totals		5	2	1			
Infiltrate, lymphocyte.							
minimal		9	12	10			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.3
 ICL607:24-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0296117w

5-MAR-04 13:35
 Page : 21
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	3 mg/kg/day	200 mg/kg/day	0 mg/kg/day	3 mg/kg/day	300 mg/kg/day
GENITOURINARY SYSTEM							
PROSTATE		(24)	(25)	(25)			
Infiltrate, lymphocyte.							
slight		5	3	5			
Score Expanded Totals		13	16	15			
Fibrosis, interstitial.							
minimal				1			
Score Expanded Totals				1			
Ectasia.							
minimal		1	1	1			
slight				1			
Score Expanded Totals		1	1	2			
Kidney normal limits		9	9	10			
SEMINAL VESICLE		(25)	(25)	(25)			
Pyknosis, epithelium.							
minimal				1			
Score Expanded Totals				1			
Metaplasia, coagulating gland.							
epithelium, focal.			1				
minimal			1				
Score Expanded Totals			1				
Infiltrate, lymphocyte.							
minimal		2	1	1			
Score Expanded Totals		2	1	1			
Dilatation, coagulating gland.							
minimal		2	3	1			
slight		1	2	3			
moderate							
Score Expanded Totals		4	10	4			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 1260726-week oral (gavage) carcinogenicity study in p51 heterozygous mice
 Study no. 0290117w

5-MAR-04 13:35
 Page : 22
 CLACS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 3 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 3 mg/kg/day	Feed w/ Iron 200 mg/kg/day
GENITOURINARY SYSTEM							
SEMINAL VESICLE		(25)	(25)	(25)			
Dilatation.							
minimal				1			
slight				1			
Score Expanded Totals				2			
Within normal limits		18	13	10			
TESTIS		(25)	(25)	(25)			
Mineralization, focal.							
minimal		1					
Score Expanded Totals		1					
Dilatation, seminiferous tubule.							
minimal				3			
slight			1	1			
Score Expanded Totals			1	4			
Degeneration, seminiferous tubule.							
minimal		1	3	1			
slight		1					
moderate				1			
Score Expanded Totals		2	3	2			
Within normal limits		22	21	10			
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)
Thickened, submucosa.							
minimal							1
Score Expanded Totals							1
Inflammation, mixed cell.							
minimal							1
Score Expanded Totals							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.3.3
 ICI67:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0270317w

5-MAR-04 13:18
 Page : 23
 PLACES 2000 V3.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
RESPIRATORY SYSTEM							
URINARY BLADDER		(25)	(25)	(25)	(25)	(25)	(25)
Infiltrate, lymphocyte, submucosa.							
minimal		2	4	4	7	5	7
slight					7	2	
moderate						2	1
marked							1
Score Expanded Totals		2	4	4	14	10	9
Hyperplasia, transitional cell, diffuse.							
minimal			1	1			
Score Expanded Totals			1	1			
Within normal limits		23	20	20	11	15	16
UTERUS							
Hyperplasia, cystic endometrial.					(25)	(25)	(25)
minimal					13	15	9
slight					1	1	1
Score Expanded Totals					14	17	9
ENDOMETRIAL STROMAL POLYP (B).					1	1	
Atrophy, endometrium.							
slight							1
Score Expanded Totals							1
Within normal limits					14	9	16
VAGINA							
Mucification.					(25)	(25)	(25)
minimal							1
slight					1		1

(B) Benign tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117W

8-NAR-04 13:35
 Page: 24
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	200 mg/kg/day
GENITOURINARY SYSTEM							
VAGINA							
Mucification.					(25)	(25)	(25)
Score Expanded Totals					1		3
Hyperplasia, epithelium.							
minimal					3	2	1
Score Expanded Totals					3	2	1
Within normal limits					21	23	21
HEMOLymphATIC SYSTEM							
ESPE MAREM							
Increase, megakaryocyte.	(25)	(25)	(25)	(25)	(25)	(25)	(25)
minimal							
Score Expanded Totals					1	1	1
Increase, fat.						1	1
minimal		1					
Score Expanded Totals		1					
Decrease, fat.							
minimal						1	1
Score Expanded Totals						2	1
Pigment, macrophage.							
minimal	10	1	4	15	2	1	1
slight	2			4			
Score Expanded Totals	12	1	4	14	2	1	1
Hyperplasia, lymphoid.							
minimal		1				2	1
Score Expanded Totals		1				2	1
HISTIOCYTIC SARCOMA (N)							
						1	1

(N) Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.1.3
 TCE607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0290117w

5-MAR-04 13:18
 Page: 25
 PLACEB 2865 VI.038

ORGAN/TISSUE Finding	TREATMENT	NUMBER OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 5 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
BONE MARROW							
Granulopoiesis, increased.		(25)	(25)	(25)	(25)	(25)	(25)
minimal				1	3	4	5
slight						1	
moderate							1
Score Expanded Totals				1	3	5	6
Fibrosis, focal.						1	
slight						1	
Score Expanded Totals						1	
Within normal limits		13	22	21	7	18	16
LYMPH NODE - AXILLARY LYMPHOMA (M)							
					(1)		
					1		
LYMPH NODE - BRONCHIAL							
Plasmacytosis							
minimal		(23)	(25)	(25)	(25)	(27)	(25)
moderate		2	1	3	2	1	1
Score Expanded Totals		2	1	3	2	1	2
Pigment, macrophage							
minimal		6	13	6	12	12	9
slight		5	3	1	4	3	1
Score Expanded Totals		9	16	7	16	15	10
Necrosis, lymphoid							
moderate				1			
Score Expanded Totals				1			
LYMPHOMA (M)							
Lymphangioectasis.					1		
minimal		3	7	5	1		1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0276117w

5-MAR-04 13:15
 Page : 26
 PLACES 2000 VI.099

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - BRONCHIAL							
Lymphangiectasis.		(23)	(25)	(25)	(25)	(23)	(25)
slight		2	2	1			
Score Expanded Totals		5	9	6	1		1
Infiltrate, mast cell, increased.							
minimal		5	9	4	2	1	4
slight		2	2	4			
Score Expanded Totals		7	11	8	2	1	4
Hypoplasia.							
minimal		6	6	2	1		
slight		1					
Score Expanded Totals		7	6	2	1		
Histiocytosis.							
minimal			1	3	1	1	
Score Expanded Totals			1	3	1	1	
HISTIOCYTIC SARCOMA (M).							
Within normal limits		7	6	7	2	2	11
LYMPH NODE - MANDIBULAR							
Extramedullary hematopoiesis.		(23)	(22)	(22)	(25)	(24)	(25)
minimal				1			
Score Expanded Totals				1			
Pigment, macrophage.							
minimal					1	1	
Score Expanded Totals					1	1	
LYMPHOMA (M).							
Lymphangiectasis.					1		
minimal				1			
slight				1			

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 12-Week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 02781170
 5-MAR-04 12:15
 Page : 27
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		a	b	c	d	e	f
		ng/kg/day	ng/kg/day	ng/kg/day	ug/kg/day	ng/kg/day	ng/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MANDIBULAR		(22)	(22)	(22)	(25)	(24)	(25)
Lymphangiectasis.							
Score Expanded Totals				2			
Infiltrate, mast cell, increased.							
minimal		5	3	7	2	1	3
slight		2	2				
Score Expanded Totals		7	6	7	2	1	3
Hyperplasia.							
minimal		2	1	1	2	1	2
slight		1		1	1		
Score Expanded Totals		3	1	2	3	1	2
Histiocytosis.							
minimal		9	6	8	9	11	12
slight		1	2	2			
Score Expanded Totals		10	8	10	9	11	12
Within normal limits		9	10	7	10	10	9
LYMPH NODE - MEDIASTINAL LYMPHOMA [M].					(1)		1
LYMPH NODE - MESENTERIC		(25)	(25)	(25)	(25)	(25)	(24)
Pigment, macrophage.							
minimal					1		
Score Expanded Totals					1		
LYMPHOMA [M].					1		
Hyperplasia.							
minimal		3		1			
Score Expanded Totals		3		1			

[M] Malignant tumor
 Figures in { } represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 TCEQST, 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-MAR-04 13:35
 Page : 28
 PLACKE 2000 V1.066

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Food w/ Iron	No Supplement	Food w/ Iron	Food w/ Iron	No Supplement	Food w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	200 mg/kg/day
HEMOLYMPHATIC SYSTEM							
LYMPH NODE - MEDITERAN		(25)	(25)	(25)	(25)	(25)	(24)
Histiocytosis							
minimal		1	1	1			
slight				1			
Score Expanded Totals		1	1	2			
Within normal limits		24	24	22	23	25	24
LYMPH NODE - PANCREATIC					(1)		
Pigment, macrophage							
marked					1		
Score Expanded Totals					1		
Infiltrate, leukophil.					1		
slight					1		
Score Expanded Totals					1		
Hemorrhage					1		
moderate					1		
Score Expanded Totals					1		
LYMPH NODE - RENAL LYMPHOMA (M)					(1)		
					1		
LYMPH NODE - SUBLINGUAL LYMPHOMA (M)					(1)		
					1		
THYMUS		(24)	(25)	(25)	(25)	(25)	(25)
Hercules, medulla							
minimal				1			
Score Expanded Totals				1			

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL67:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. CR70117W

5-NAR-04 12:35
 Page: 28
 PLACES 2866 V1.06B

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
HEMOLYTHATIC SYSTEM							
THYMUS		(24)	(25)	(25)	(25)	(25)	(25)
Necrosis, cortex.							
marked				1			
Score Expanded Totals				1			
Inflammation, artery.							
moderate							1
Score Expanded Totals							1
Hyperplasia, epithelial.							
minimal				1			
Score Expanded Totals				1			
Hyperplasia, lymphoid.							
minimal		2	2	2			
Score Expanded Totals		4	4	4			
HISTIOCYTIC SARCOMA (M)							
Cyst.					1		
minimal				1	1		
slight		1	2	1	1		
Score Expanded Totals		1	2	1	2		
Atrophy.							
moderate							1
Score Expanded Totals							1
within normal limits		19	21	20	23	25	24
SPLEEN		(24)	(25)	(25)	(25)	(25)	(25)
Extramedullary hematopoiesis.							
increased.				1	5	2	6
minimal							
slight					2		1

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 12L687: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117w
 5-MAR-03 13:35
 Page : 30
 PLANS 2600 V3.001

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day
HEMOLYMPHATIC SYSTEM							
SPLLEN		(24)	(25)	(25)	(25)	(25)	(25)
Extramedullary hematopoiesis, increased.							
marked					1		
Score Expanded Totals				1	1		1
Pigment, melanocyte.						2	7
minimal			2	3	1		
slight			1				
moderate	1						
Score Expanded Totals	1		3	3	1		
Pigment, macrophage, increased.						5	6
minimal		2		1	7		2
slight					12		5
Score Expanded Totals	2			1	17	5	7
Neurosis, lymphoid.							
minimal				1			
Score Expanded Totals				1			
Hypertrophy, lymphoid.							
minimal		1		2	1	1	
Score Expanded Totals		1		2	1	1	
HYPERTYPIC SARCOMA (M).							
Depletion, lymphoid.							
minimal				1			1
Score Expanded Totals				1			1
Deficiency.							
minimal	1						
slight	1						
Score Expanded Totals	2						

[M] Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 R2L897:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 study no. 0278117W

5-MAR-24 11:35
 Page : 21
 PLACE3 2005 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Diad w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
b	b	b	b	b	b	b	
		mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
HEMOLYMPHATIC SYSTEM							
SPLEEN		(24)	(25)	(24)	(25)	(25)	(25)
Congestion,							
moderate				1			
Severe Expanded Totals				1			
Within normal limits		18	20	19	8	19	11
SYSTEMIC/LYMPH SITE		(25)	(25)	(25)	(25)	(25)	(25)
MALT CELL LYMPH (M).				1			
LYMPHOMA (M)					1		
HISTIOCYTIC SARCOMA (M)					1		
Within normal limits		25	25	24	23	25	25
INTEGUMENTARY SYSTEM							
CLITORAL GLAND					(20)	(22)	(21)
Inflammation, mixed cell,							
minimal					6	5	2
Severe Expanded Totals					6	5	2
Ectasia,							
minimal							1
Severe Expanded Totals							1
Within normal limits					14	17	19
MAMMARY GLAND		(13)	(21)	(11)	(25)	(25)	(25)
Hyperplasia, duct,							
minimal						1	
Severe Expanded Totals						1	
Within normal limits		3	2	1	25	24	25

(B) Benign tumor
 (M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0290117w

5-MAR-04 13:35
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 PLANS 2000 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Food w/ Iron	No Supplement	Food w/ Iron	Food w/ Iron	No Supplement	Food w/ Iron
		5 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
INFERIORITY SYSTEM							
PROSTATE GLAND							
Inflammation, mixed cell.		(25)	(25)	(25)			
minimal		2		1			
Score Expanded Totals		2		1			
Inflammation, chronic.							
minimal		2	1	6			
Score Expanded Totals		2	1	6			
Hypertrophy, epithelium, duct.							
minimal		1	1	1			
Score Expanded Totals		1	1	1			
Hypertrophy, acinar, focal.							
minimal		1	1				
Score Expanded Totals		1	1				
Stenosis							
minimal		2	4	7			
slight			1	1			
Score Expanded Totals		2	5	8			
Atrophy							
minimal		1	2	1			
slight				1			
Score Expanded Totals		1	2	2			
Within normal limits		16	19	19			
SKIN							
Mineralization, cartilage, pinna, focal.		(25)	(25)	(25)	(25)	(25)	
minimal				1	1	2	
slight						1	
Score Expanded Totals				1	1	3	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.3
 TCE607: 26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0279117w

5-MAR-04 12:39
 Page: 13
 CLACES 2600 V1.008

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	3 mg/kg/day	200 mg/kg/day	0 mg/kg/day	6 mg/kg/day	300 mg/kg/day
INTEGUMENTARY SYSTEM							
SKIN		(25)	(25)	(25)	(25)	(25)	
Inflammation, mixed cell, subcutis, minimal						1	
Score Expanded Totals						1	
Inflammation, subcutis, pinna, focal, minimal				2			
slight				2		3	
moderate				1			
Score Expanded Totals				5		3	
Infiltrate, lymphocyte, fat, minimal		2	1	1			
slight				1			
Score Expanded Totals		2	1	2			
Hyperplasia, cartilage, pinna, focal, minimal							
slight				2	2	1	
Score Expanded Totals				2	2	1	
Hyperplasia, epidermis, pinna, focal, minimal							
slight						1	
Score Expanded Totals						1	
Granulation tissue, dermis, focal, slight				1			
moderate				1			
Score Expanded Totals				2		1	

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 5.2.3
 IC5651; 24-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 027611w

5-MAR-04 13:35
 Page : 34
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPE FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day
INTEGUMENTARY SYSTEM							
SKIN		(25)	(25)	(25)	(25)	(25)	(25)
Fibrosis, cartilage, pinna, focal.							
minimal		1	1	1	2	4	
Score Expanded Totals		1	1	1	2	4	
Fibrosis, dermis, pinna, focal.							
minimal		1	1	2	1	2	2
slight			1	2	1	2	2
moderate						1	1
Score Expanded Totals		1	2	2	2	2	5
Cyst, inclusion, epidermal.				1			
moderate				1			
Score Expanded Totals				1			
Atrophy, hair follicles.							
minimal				1	1	1	1
moderate		1					
Score Expanded Totals		1		1	1	1	1
Abscess, epidermis.							
minimal				1			1
moderate				1			
marked				1			
Score Expanded Totals				1			1
Within normal limits		21	22	17	20	17	20
MUSCULOSKELETAL SYSTEM							
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)
Proliferated, periosteum, focal.							
minimal			1				
Score Expanded Totals			1				

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 1.2.3
 ICL667:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-MAR-04 13:15
 Page : 35
 PLACE 2003 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron (No Supplement)	Feed w/ Iron (No Supplement)	Feed w/ Iron (No Supplement)	Feed w/ Iron (No Supplement)	Feed w/ Iron (No Supplement)	Feed w/ Iron (No Supplement)
	0 mg/kg/day	205 mg/kg/day	0 mg/kg/day	205 mg/kg/day	0 mg/kg/day	205 mg/kg/day	
MUSCULOSKELETAL SYSTEM							
BONE - FEMUR/TIBIA		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, synovium, moderate		1					
Score Expanded Totals		1					
Within normal limits		24	24	25	25	25	25
BONE - SPERMUM		(25)	(25)	(25)	(25)	(25)	(25)
Within normal limits		25	25	25	25	25	25
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)
Regeneration, myofiber, focal, minimal		1	1	10	1	1	3
Score Expanded Totals		1	1	10	1	1	3
Regeneration, myofiber, near insertion at patella, minimal		1					
Score Expanded Totals		1					
Necrosis, fibrinoid, artery, slight							1
Score Expanded Totals							1
Mineralization, myofiber, focal, minimal			1	5			
Score Expanded Totals			1	5			
Inflammation, artery, moderate							1
Score Expanded Totals							1
Inflammation, mixed cell, focal, near insertion at patella, minimal		1					
Score Expanded Totals		1					

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICR1607:24-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w

5-MAR-04 13:15
 Page: 36
 BLACBS 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	NoSupplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	NoSupplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
MUSCULOSKELETAL SYSTEM							
MUSCLE		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell, focal.							
minimal		1			1	1	
slight					1		
Score Expanded Totals		1			2	1	
Inflammation, focal, chronic.			1	2			
minimal			1	2			
Score Expanded Totals			1	2			
Fibrosis, focal, near insertion at patella.							
slight		1					
Score Expanded Totals		1					
Fibrosis, focal.							
minimal					1		
Score Expanded Totals					1		
Degeneration, myofiber, focal.				4			2
minimal				4			2
Score Expanded Totals				4			2
Within normal limits		23	21	13	22	24	20
NERVOUS SYSTEM							
BRAIN		(25)	(25)	(25)	(25)	(25)	(25)
Vacuolation, nucleus oblongata, focal.							
minimal				1			
Score Expanded Totals				1			
Mineralization, thalamus, focal.							
minimal		5	1	2			
Score Expanded Totals		5	2	2			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.2
 IEL607: 26-week oral (gavage) carcinogenicity study in B63 heterozygous mice
 Study no. 0276317w

5-MAR-04 15:35
 Page : 37
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	200 mg/kg/day
NERVOUS SYSTEM							
BRAIN							
Mineralization, meninges, focal, slight		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals				1			
Inflammation, artery, meninges, moderate				1			
Score Expanded Totals							1
Dilatation, ventricles, minimal		1		2		1	
Score Expanded Totals		1		2		1	
Within normal limits		19	23	21	25	24	24
NERVE - SCIATIC							
Within normal limits		(25)	(25)	(25)	(25)	(25)	(25)
		25	25	25	25	25	25
SPINAL CORD							
Nakase, focal, minimal		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals							1
Diiodosis, focal, minimal							1
Score Expanded Totals							1
Cyst, meninges, minimal				1			
Score Expanded Totals				1			
Within normal limits		25	25	24	25	25	24

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.2.3
 ICL687:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0276117w

4-MAR-04 13:35
 Page : 78
 PLACEB 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 mg/kg/day	0 mg/kg/day	200 mg/kg/day	0 mg/kg/day	0 mg/kg/day	300 mg/kg/day
RESPIRATORY SYSTEM							
LARYNX							
Inflammation, mixed cell, minimal							(1)
Score Expanded Totals							1
LUNG							
Swelling, epithelial cell, bronchiolar, moderate		(25)	(25)	(25)	(25)	(25)	(25)
Score Expanded Totals				1			
Inflammation, granulocytous, focal, minimal							1
Score Expanded Totals							1
Inflammation, mixed cell, focal, minimal				1			
Score Expanded Totals				1			
Inflammation, pleura, mixed cell, focal, minimal					1		
Score Expanded Totals					1		1
Inflammation, perivascular, minimal							1
Score Expanded Totals							1
Infiltrate, lymphocyte, pleura, focal, minimal		1	1	2	2	1	2
Score Expanded Totals		1	1	2	2	1	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 2.4.2
 ICI687:25-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. Q198117

5-MAR-04 12:15
 Page: 18
 BLACS 2000 V1.032

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 200 mg/kg/day	Feed w/ Iron 0 mg/kg/day	No Supplement 0 mg/kg/day	Feed w/ Iron 300 mg/kg/day
RESPIRATORY SYSTEM							
LUNG		(25)	(25)	(25)	(25)	(25)	(25)
Hyperplasia, lymphoid tissue.							
minimal		2	2	0	2	4	3
slight				1			1
Score Expanded Totals		3	2	1	2	4	4
Hyperplasia, bronchioalveolar, focal.							
minimal				2			
Score Expanded Totals				2			
Histiocytosis, alveolar, focal, with crystals.							
minimal				2			
Score Expanded Totals				2			
Histiocytosis, alveolar, focal.							
minimal				1			
slight			1	1			
Score Expanded Totals			1	2			
HISTIOCYTIC SARCOMA (M)							
Fibrosis, pleura, focal.					1		
minimal					1		
Score Expanded Totals					1		
Fibria, pleura.							
moderate					1		
Score Expanded Totals					1		
Crystal, hemosiderin, bronchiole.							
minimal							
Score Expanded Totals							
Within normal limits		20	21	14	26	19	18

(M) Malignant tumor
 Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.3
 ICL607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 9276117a

5-MAR-06 13:35
 Page : 40
 PLACES 2000 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Food w/ Iron	No Supplement	Food w/ Iron	Food w/ Iron	No Supplement	Food w/ Iron
g	0	200	0	0	300	300	
		mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
RESPIRATORY SYSTEM							
TRACHEA		(25)	(25)	(25)	(25)	(25)	(25)
Inflammation, mixed cell.							
minimal			1	1			
Score Expanded Totals			1	1			
Inflammation, submucosa, chronic.							
minimal		1	1	1	1		1
slight						1	
Score Expanded Totals		1	1	1	1	1	1
Within normal limits		24	23	21	24	24	23
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Swelling, epithelial cell, cornea, focal.							
minimal				1			
Score Expanded Totals				1			
Pyknosis, lens.							
minimal		25	24	22	18	19	20
slight			1	2			4
moderate							1
Score Expanded Totals		25	25	24	18	19	25
Degeneration, fiber, lens.							
minimal		17	7	19	5	6	7
slight		4	5	7	3	2	5
moderate				1			1
marked							1
Score Expanded Totals		21	12	21	8	8	13
Cataract.							
minimal							1

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.2.2
 126607:26-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0270117w
 5-MAR-04 12:25
 Page : 41
 PLACEB 2666 71.002

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Food w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Food w/ Iron 200 ng/kg/day	Food w/ Iron 0 ng/kg/day	No Supplement 0 ng/kg/day	Food w/ Iron 300 ng/kg/day
SPECIAL SENSE ORGANS							
EYE		(25)	(25)	(25)	(25)	(25)	(25)
Cataract							
slight							1
moderate							1
marked							1
Score Expanded Totals				1	7	6	4
Within normal limits							
HABERMAN GLAND		(25)	(25)	(25)	(25)	(25)	(25)
Pigment, increased							
minimal				1			
Score Expanded Totals				1			
Infiltrate, lymphocyte							
minimal		5	3	5	8	7	6
slight		1		1			1
Score Expanded Totals		6	3	6	8	7	7
Hyperplasia, focal							
minimal		1					
Score Expanded Totals		1					
Dilatation, acini							
minimal		5	2	2	1		
Score Expanded Totals		5	2	2	1		
Within normal limits		14	20	18	16	18	18
LACRIMAL GLAND		(25)	(25)	(25)	(24)	(24)	(24)
Metaplasia, focal							
minimal			4	3		1	
slight							1
Score Expanded Totals			4	3		1	2

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Table 3.1.3
 ICL687: 16-week oral (gavage) carcinogenicity study in p53 heterozygous mice
 Study no. 0278117a
 5-NSR-04 13:15
 Page : 42
 BLACS 2880 V1.000

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		Feed w/ Iron	No Supplement	Feed w/ Iron	Feed w/ Iron	No Supplement	Feed w/ Iron
		0 ng/kg/day	6 ng/kg/day	228 ng/kg/day	0 ng/kg/day	0 ng/kg/day	100 ng/kg/day
SPECIAL SENSE ORGANS							
LACRIMAL GLAND							
Infiltrate, lymphocyte.		(25)	(25)	(25)	(24)	(24)	(24)
minimal		15	16	11	13	11	12
slight		10	7	11	4	3	4
moderate							1
Score Expanded Totals		25	23	22	17	14	17
Cyst.							
minimal				1			
Score Expanded Totals				1			
Atrophy, acinar.							
minimal		9	14	8	5	2	4
slight		15	7	16	1	1	2
moderate		1	3	3			
marked							1
Score Expanded Totals		25	24	27	6	3	7
Within normal limits				1	6	9	5

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

*** Listing Complete ***

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/s/

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9/28/2005 12:30:03 PM
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