

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-232

PHARMACOLOGY REVIEW(S)

**PHARMACOLOGY/TOXICOLOGY COVER SHEET**

11-14-01

NDA 21-232

Review number: 2

Sequence number/date/type of submission: "complete response to May 3, 2001 approvable"

Information to sponsor: Yes (X) No ( )

Sponsor and/or agent: Swedish Orphan, AB; R& R Registrations (US agent)

Manufacturer for drug substance : not specified

Reviewer name: Karen Davis-Bruno; Ph.D.

Division name: DMEDP

HFD #: 510

Review completion date: 11/13/01

Drug:

Trade name: Orfadin

Generic name (list alphabetically): nitisinone

Relevant INDs/NDAs/DMFs: IND —

Drug class: triketone, enzyme inhibitor, originally an herbicide

Indication: hereditary tyrosinemia type 1

Clinical formulation: 2, 5, 10 mg capsules with excipients: pre-gelatinized starch, gelatin, titanium dioxide

Route of administration: oral

Proposed use: treatment of hereditary tyrosinemia type 1 (pediatric) with dosing individualized

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

**APPEARS THIS WAY  
ON ORIGINAL**



***TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW***

**I. PHARMACOLOGY:..... 1**

**II. SAFETY PHARMACOLOGY:..... 1**

**III. PHARMACOKINETICS/TOXICOKINETICS: ..... 1**

**IV. GENERAL TOXICOLOGY:..... 1**

**V. GENETIC TOXICOLOGY:..... 1**

**VI. CARCINOGENICITY: ..... 1**

**VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:..... 1**

**VIII. SPECIAL TOXICOLOGY STUDIES: ..... 1**

**IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:..... 1**

**X. APPENDIX/ATTACHMENTS: ..... 1**

**APPEARS THIS WAY  
ON ORIGINAL**

***PHARMACOLOGY/TOXICOLOGY REVIEW***

- I. PHARMACOLOGY:** New data has not been submitted
- II. SAFETY PHARMACOLOGY:** New data has not been submitted
- III. PHARMACOKINETICS/TOXICOKINETICS:** New data has not been submitted
- IV. GENERAL TOXICOLOGY:** New data has not been submitted
- V. GENETIC TOXICOLOGY:** New data has not been submitted
- VI. CARCINOGENICITY: NEW DATA HAS NOT BEEN SUBMITTED**
- VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:** New data has not been submitted
- VIII. SPECIAL TOXICOLOGY STUDIES:** New data has not been submitted
- IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:** not applicable see recommendations
- X. APPENDIX/ATTACHMENTS:** not applicable

**APPEARS THIS WAY  
ON ORIGINAL**

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

/s/

-----  
Karen Davis-Bruno

11/14/01 08:49:24 AM

PHARMACOLOGIST

Comments to the sponsor re:reprotox protocols and timeline

**APPEARS THIS WAY  
ON ORIGINAL**

**MEMORANDUM**

DATE: March 9, 2001

FROM: Kenneth L. Hastings, Dr.P.H.  
Acting Associate Director for Pharmacology/Toxicology, ODE II

TO: NDA 21-232  
Orfadin (nitisinone) capsules

I have reviewed the Pharmacology/Toxicology data, related documents, and proposed label for Orfadin and concur that the product is approvable. The product label accurately reflects the pharmacology/toxicology data; however, I propose the following change in the *Carcinogenesis, Mutagenesis, Impairment of Fertility* section:

*Studies in animals have not been conducted to evaluate the carcinogenic potential of nitisinone. Nitisinone was not mutagenic in the Ames test. A study in rats given single oral doses of 100 mg/kg (12 times the recommended clinical dose, based on relative body surface area) demonstrated reduced litter size, decreased pup weight at birth, and decreased survival of pups after birth.*

I also propose the following change in the *Pregnancy* section:

***Pregnancy Category C.*** Adequate reproductive toxicity studies ~~have~~ have not been conducted. It is not known if nitisinone can produce harm to the fetus if administered to a pregnant woman. Nitisinone should be administered to a pregnant woman only if clearly needed.

[  
] . Reproductive toxicity studies should be conducted as part of  
Phase IV commitments.

---

Kenneth L. Hastings, Dr.P.H.

**APPEARS THIS WAY  
ON ORIGINAL**



12/15/00

### REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

**KEY WORDS:** tyrosine, eyes, ocular toxicity, corneal damage, opacities, alterations, lenticular lesions, liver, kidneys

**Reviewer Name:** David H. Hertig

**Division Name:** Metabolism and Endocrine Drug Products

**HFD#:** 510

**Review Completion Date:** 5 Dec 00

**NDA number:** 21-232

**Serial number/date/type of submission:** N-000; 27 Dec 99; Resubmission 7 Sep 00

This product has been granted an Orphan Drug Designation (#95-890).

**Information to sponsor:** Yes (X) No ( ) See Labeling p. 47

**Sponsor (or agent):** Swedish Orphan, AB, Drottninggatan 98, Stockholm, Sweden SE-111 60

[Authorized U.S. Agent: R & R Registrations, P.O. Box 262069, San Diego, California 92196-2069]

**Manufacturer:** \_\_\_\_\_ bulk drug substance:

**Producer of Drug Product:** Apoteket AB, Production and Laboratories, Importgatan 20, Hisingbacka, S-401 20, Gothenburg, Sweden

**Drug:**

**Code Name:** SCO-0735 (NTBC and R-82735)

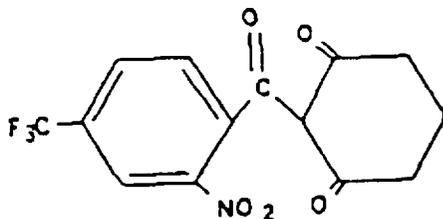
**Generic Name:** Nitisinone

**Trade Name:** Orfadin

**Chemical Name:** 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione or 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione, IUPAC

**Molecular Formula/ Molecular Weight:** C<sub>14</sub>H<sub>10</sub>F<sub>3</sub>NO<sub>5</sub> · Relative mass 329.23

**Structure:**



2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione

**Relevant INDs:** IND \_\_\_\_\_ (Swedish Orphan AB) Orphan Drug 95-890

**Drug Class:** Triketone; enzyme inhibitor Originally intended use as a herbicide.

**Indication:** Hereditary tyrosinemia type 1 (HT-1)

**Clinical formulation:** Capsules 2, 5, 10 mg

Each capsule contains, 2 mg, 5 mg, 10 mg Nitisinone, plus pregelatinised starch. The capsule shell is gelatin and titanium dioxide and the imprint is an iron oxide.

**Route of administration:** Oral Capsule

**Proposed Use:**

Treatment should be initiated by a physician experienced in the treatment of hereditary tyrosinemia type 1. The majority of patients treated with Nitisinone are pediatric.

The dose of ORFADIN™, Nitisinone should be adjusted individually. An initial dose of 0.5 mg/kg body weight twice daily is recommended. The patient should be treated with a strict low protein diet, supplemented with an amino acid formula deficient in phenylalanine and tyrosine.

*Dose adjustment:* Nitisinone treatment should block the flux through the tyrosine degradation pathway at the level of 4-hydroxyphenylpyruvate dioxygenase. Treatment should lead to a normalized porphyrin metabolism i.e. a normal erythrocyte porphobilinogen synthetase activity and 5-aminolevulinic acid urine excretion. Succinylacetone should not be detectable in urine or plasma. If the biochemical parameters (except plasma succinylacetone) are not normalized one month after start of Nitisinone treatment, the Nitisinone dose should be increased to 0.75 mg/kg body weight twice daily.

For plasma succinylacetone, it may take up to three months before the level is normalized after start of Nitisinone treatment. Especially in infants a dose of up to 1 mg/kg body weight twice daily might be needed once the liver function has improved.

1 mg/kg body weight twice daily should be considered as a maximal dose for all patients.

**Disclaimer – use of sponsor's material:** Note some material may be taken directly from sponsor's submission.

**Introduction and drug history:**

This drug was originally developed as a potential herbicide. When it was later discovered that NTBC was associated with toxic ocular effects in rats and dogs the development of the chemical was stopped

[Mice, rabbits and monkeys appear to be fairly resistant to NTBC-induced ocular opacity.]

NTBC has been found to be a potent inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) and to cause an elevation in plasma tyrosine. It was also found to prevent accumulation of electrophilic compounds and their decarboxylated products known to react with SH-groups and be responsible for liver and kidney injuries in patients with hereditary tyrosinemia type I a serious and life-threatening disorder with a liver transplant being the only hope at present. The biochemical defect in hereditary tyrosinemia type 1 is a deficiency of fumarylacetoacetase, which is the final enzyme of the tyrosine catabolic pathway. ORFADIN™, Nitisinone (NTBC) inhibits the activity of 4-hydroxyphenylpyruvate dioxygenase, which is the second enzyme of the same pathway, and as a result the accumulation of toxic tyrosine metabolites in patients with hereditary tyrosinemia type 1 is reduced or prevented. Orfadin treatment leads to decreased urinary excretion of succinylacetone, increased plasma tyrosine concentration and increased urinary excretion of phenolic acids.

Orfadin was granted Orphan Drug status (# 95-890) for use in hereditary tyrosinemia type I for which the total world population is thought to be in the order of less than 400 patients half of which have probably already received Orfadin in clinical trials. NTBC is reported to improve survival and currently there is no pharmacologic therapy approved to treat this condition. Although not all of the preclinical studies are complete the sponsor was permitted to file an NDA for this specific (potentially fatal) indication with the preclinical data on hand.

See Tyrosine Degradation Pathway this review page 5.

**Studies reviewed within this submission:** See Table of Contents next page (p. 3).

<b>TABLE OF CONTENTS</b>	<b>PAGE NUMBER*</b>
<b>PHARMACOLOGY/PHARMACODYNAMICS/PHARMACOKINETICS</b>	
— R/1028: SC-0735 Pharmacological Evaluation Report 90-08-17	IND - 4
— /R/983: Inhibition of Rat Liver 4-hydroxyphenyl-pyruvate dioxygenase by SC-0735 in Vitro; Characterization and Kinetic Parameter Estimation Report 89-12-13	NDA - 6
— /R/1155: Tissue Distribution of SC-0735 and Its Effect on Enzymes Involved in Tyrosine Catabolism in the Rat. Report 94-03-31	NDA - 6
— /R/1187: Tissue Distribution of SC-0735 and Its Effect on Enzymes Involved in Tyrosine Catabolism in the Mouse. Report 94-05-17	IND - 4
1995-10-10: NTBC Pharmacokinetics in the Rat. Report 1995-10-10	NDA - 11
T-13585: SC-0735 Metabolism Probe in Rats Report 88-06-13	NDA - 11
<b>SINGLE DOSE TOXICITY STUDIES</b>	
T-12214: Stage 1 Acute Toxicity Test Battery for R-82735 Report 86-03-21	IND - 5 NDA - 15
<b>REPEATED DOSE TOXICITY STUDIES</b>	
T-12949: 6-Week Range-Finder Probe With SC-0735 in Rats — Report 88-02-17	IND - 5
T-12984: Integrated 13-week Subchronic and Chronic Toxicity/oncogenicity Study [oncogenicity portion aborted] With SC-0735 in rats. Report 96-05-08 — /T/2906: SC-0735 Chronic Toxicity in Rats – Histopathological Examination of Selected Tissues – Individual Animal Data Report — /P/4710	IND - 6 NDA - 15
T-13249: Recovery Study With SC-0735 in Rats — Report 88-06-27	IND - 6
T-13255: Three-month Route of Exposure Study with SC-0735 in Rats. — Report 89-02-20	IND - 6
T-12963: Six-week Dietary Range Finding Probe with SC-0735 in Mice — Report 87-06-23	IND - 7
T-13005: Oncology Study (aborted) with SC-0735 in Mice — Report 88-08-31	IND - 7
T-12965: Dog Range Finder with SC-0735 (Final) — Report 87-07-27	IND - 7
T-13004: Three-month Oral toxicity Study with SC-0735 in Beagle Dogs — Report 89-03-23	IND - 8
T-13256: Three-month Ocular Toxicity Probe with SC-0735 in Rabbits — Report 88-12-13	IND - 8
T-13587: Range Finding/13-week Ocular Toxicity Study with SC-0735 in Primates — Report 89-11-06	IND - 8
<b>REPRODUCTIVE/TERATOLOGY STUDIES:</b>	
T-12725: A Fertility Probe in CD Rats with SC-0735 Report 87-08-21	IND - 9
T-12724: A Teratology Screen in CD Rats with SC-0735 Report 87-08-21	IND - 9
T-12935: A Range Finding Teratology Probe in CD Rats with SC-0735 Technical Report 88-07-19	NDA - 20
T-13247: SC-0735 A Cross-fostering Reproduction Probe in Rats Report 89-12-15	NDA - 21

**GENETIC TOXICOLOGY:**

T-12608: Mutagenicity Evaluation in Salmonella Typhimurium of SC-0735 Report 85-10-21	IND - 10
— /P/6300: NTBC Purified: Bacterial Mutation Assay in S Typhimurium and E.Coli Report 99-07-27	NDA - 31
— /P/6301: NTBC Unpurified: Bacterial Mutation Assay in S. Typhimurium and E. Coli Report 99-07-27	NDA - 35
T-12609: Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Forward Mutation Assay. Report 86-05-27	IND - 10
T-12610: Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytogenic Assay. Report 86-04-03	IND - 10
T-12611: Morphological Transformation of BALB/3T3 Cells Report 85-12-05	IND - 11
T-12611: Morphological Transformation of BALB/3T3 Cells, Activation Report 88-04-12	NDA - 38
T-12664: Effects of SC-0735 on Human Fibroblast DNA Report 85-11-05	IND - 11
T-12771: Mutagenicity Evaluation in Bone Marrow Micronucleus Report 86-04-03	IND - 11

**SPECIAL STUDIES/OTHER INFORMATION**

— /R/1192: SC-0735: Study to Investigate the Morphological Aspects of Ocular Toxicity in Rats and the Potential for Recovery Report 94-02-07	IND - 12
— /R/1198: SC-0735: Study to Investigate the Morphological Development of the Corneal Lesion in the Rat by Light Microscopy Report 94-03-09	IND - 12
— /R/1201: The Effects of L-tyrosine Supplemented Low Protein Diet in the Rat Report 94-04-12	IND - 13
— /R/1206: SC-0735 Induced Tyrosinemia in the Dog, Rabbit, and Rhesus Monkey Report 94-08-10	IND - 14
Letter: ————— Effects of Compound 0735 on Brain (Striatal Region) Biogenic Amino Levels	NDA - 38

**REPRINT**

Lindstedt S., et al: Treatment of Hereditary Tyrosinemia Type I by Inhibition of 4-hydroxyphenylpyruvate dioxygenase. The Lancet, Vol. 340, 813-817, Oct 3, 1992	IND - 2/3
--	-----------

**Overall Summary/Comments-Evaluation:**

NDA - 40/44

**Labeling:**

NDA - 47

**Recommendation:**

NDA - 47

**Information to Sponsor:**

NDA - 47

\* Page Numbers: IND = Pharm. Review of IND — dtd. 26 Jan 95 - attached  
NDA = This NDA (21-232) Review

Tyrosine degradation and porphyrin synthesis: From Pharmacology Review of IND —, 26 Jan 95 attached.

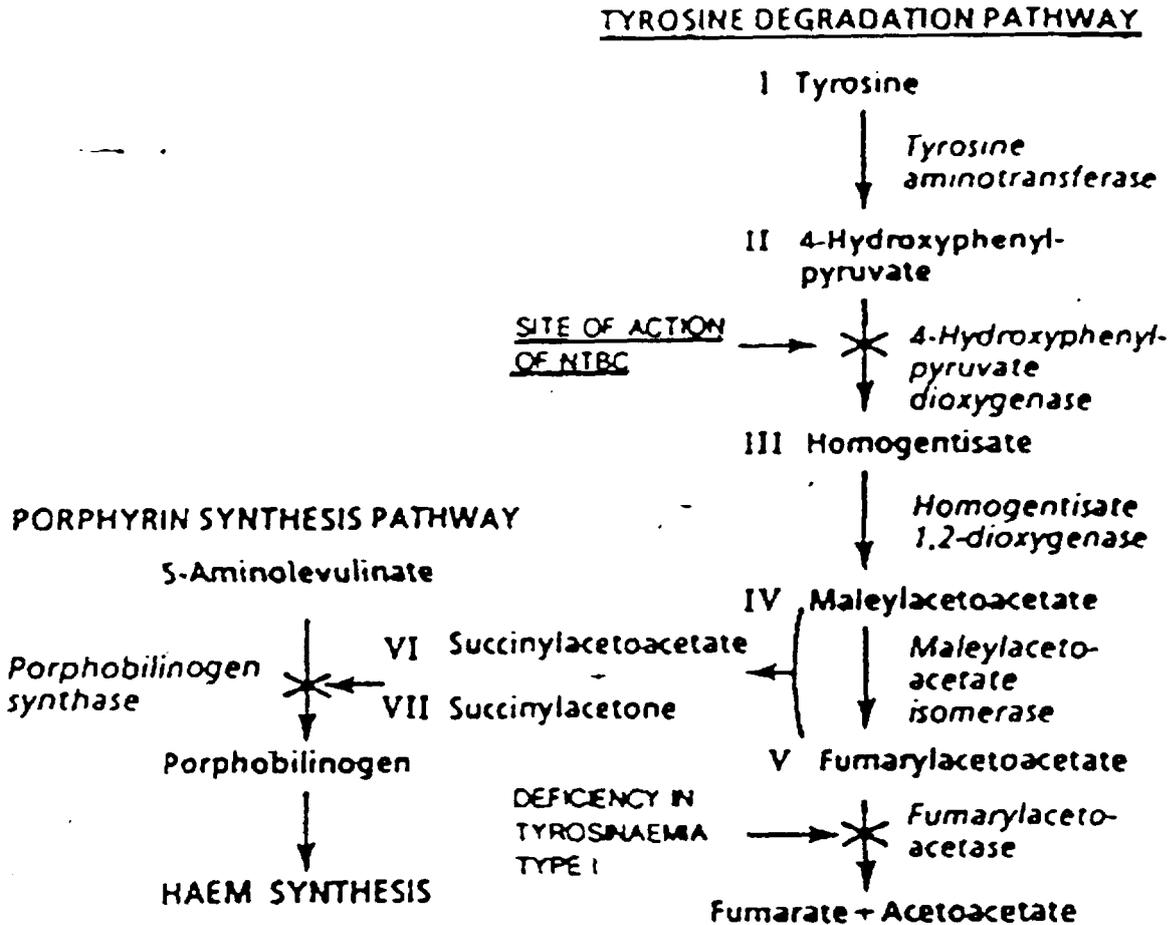


Fig 1—Tyrosine degradation and porphyrin synthesis.

**APPEARS THIS WAY  
ON ORIGINAL**

**PHARMACODYNAMICS/PHARMACOKINETICS**

Additional tests not in the Review of referenced IND — dtd. 6 Jan 95.

Inhibition of Rat Liver 4-hydroxyphenyl-pyruvate Dioxygenase by SC-0735 In Vitro; Characterization and Kinetic Parameter Estimation, Report 89-12-13.

Report — /R/983 dtd. 13 Dec 89. Vol. 1.7 p. 001

It is reported that certain triketones are known to cause an eye lesion in rats. SC-0735 has been found to cause increases in blood tyrosine levels in vivo and it has been suggested that the eye lesion is related to this excess tyrosine. Mathematical modeling was used to characterize the kinetics of inhibition of rat liver 4-hydroxyphenylpyruvate dioxygenase (the key step in the catabolism of tyrosine) by SC-0735 in vitro.

It was concluded that SC-0735 is an irreversible/tight-binding inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase with a second order rate constant for this inhibition with the rate of inactivation of enzyme being proportional to the concentrations of both free active enzyme and SC-0735.

Tissue Distribution of SC-0735 And Its Effect on Enzymes Involved in Tyrosine Catabolism In The Rat, Report 94-03-31.

Report — /R/1155 dtd. 31 Mar 94. Vol. 1.7 p. 021

Male Alpk:ApfSD (Wistar-derived) rats 5-6 weeks old were usually studied in groups of 4 animals per dose level. Radiolabeled [<sup>14</sup>C]-SC-0735 was administered at 30 μmol/kg (50 μCi/kg) or 0.3 μmol/kg (9 μCi/kg). Tissues collected were liver, kidneys, lungs, brain, eyes, intra and extra-orbital lachrymal glands and Harderian glands. Statistical analysis was by Student's t-test and p values <0.05 were considered significant.

SC-0735 produces a dose-dependant tyrosinemia that can persist for several days at a dose of 30 μmol/kg (10 mg/kg). At this dose, the concentration of tyrosine in ocular fluid markedly increases and persists for several days. Repeated, but not single, administration produces ocular toxicity in rats. The incidence of lesions in rats was about 40% at 0.3 μmol/kg/day and about 80% at 30 μmol/kg/day — Study XR1560 (1989). [It is reported that rats fed a low protein diet supplemented with tyrosine also develop a marked and sustained tyrosinemia and ocular lesions (Harper *et al.*, 1970, *J. Biol. Chem.* **239** 3821-3825; Rich *et al.*, 1973, *Exp. Eye Res.* **17** 87-97; Goldsmith, 1983, Ed. J B Stanbury *et al.* McGraw-Hill pp287-299.) - ocular lesions, which resemble those, produced by SC-0735.]

Hepatic enzyme analysis involved with tyrosine catabolism showed that 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) activity is inhibited soon after dosing with either 0.3 or 30 μmol/kg SC-0735 with slow recovery of activity. Tyrosine aminotransferase activity (the first and rate limiting enzyme in tyrosine catabolism) was induced about 2-fold in the liver at 24 hrs. (presumably a response to the increased tyrosine levels – resulting in an increase in the metabolism of tyrosine and thereby increasing its elimination as 4-hydroxyphenylpyruvic acid), while the activity of homogentisic acid oxidase showed little or no effect.

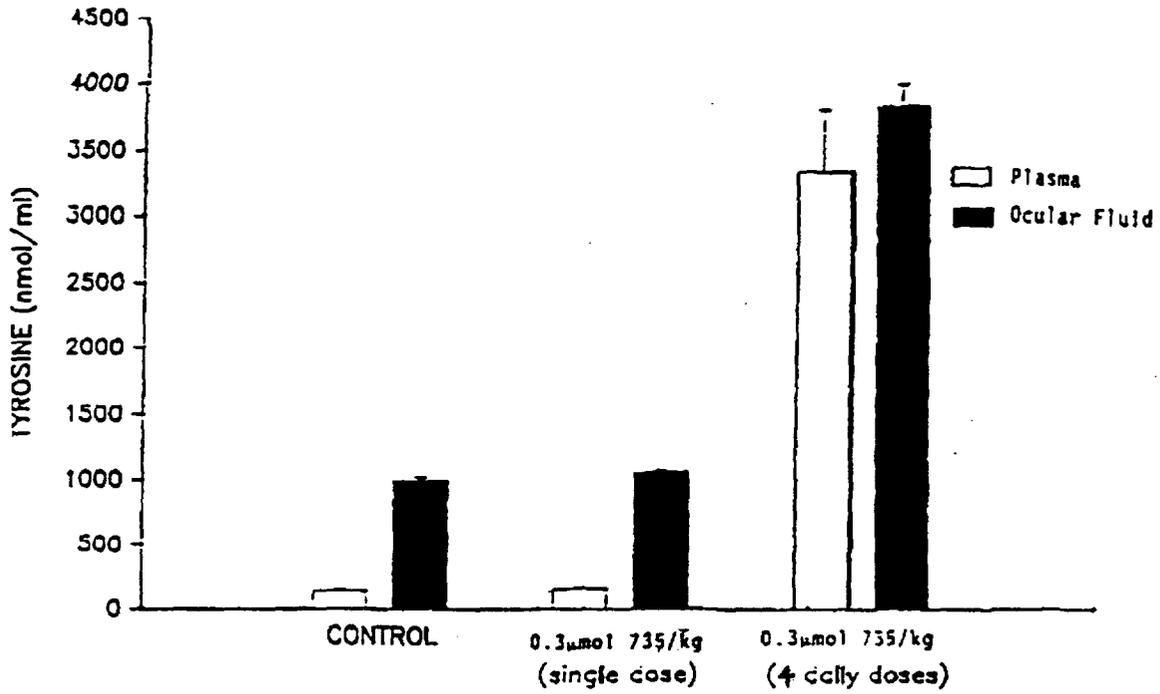
[<sup>14</sup>C]-SC-0735 radioactivity does not selectively accumulate in the rat eye or cornea, but liver and kidney retain the radiolabel, which is most marked at 0.3 μmol/kg where the tissue concentration 4 days (ca 50%) after dosing is similar to that at 1 hour. A similar amount of radiolabel can be measured in the liver and kidneys after 30 μmol/kg suggesting that there may be a saturable compartment in the cytosol. Retention of radiolabel in the liver and kidney may be due to binding to 4-HPPD, which have the body's highest concentration of 4-HPPD.

No radiolabel retention was detected in the cornea, which is the site of toxicity; the radiolabel is not readily lost from the Harderian gland, but the profile of retention and elimination is different from that of the kidney or liver. [See following tables pages 9, 10.]

Thus, retention of the SC-0735 radiolabel in the liver and kidney causes a marked inhibition of 4-HPPD leading to a dose-dependent increase in tyrosine concentration in plasma and ocular fluid.

Sponsor's Figure: Vol. 1.7/039

PLASMA AND OCULAR FLUID TYROSINE CONCENTRATIONS IN THE RAT  
FOLLOWING A SINGLE OR FOUR DAILY DOSES OF SC-0735 AT 0.3 $\mu$ mol/kg

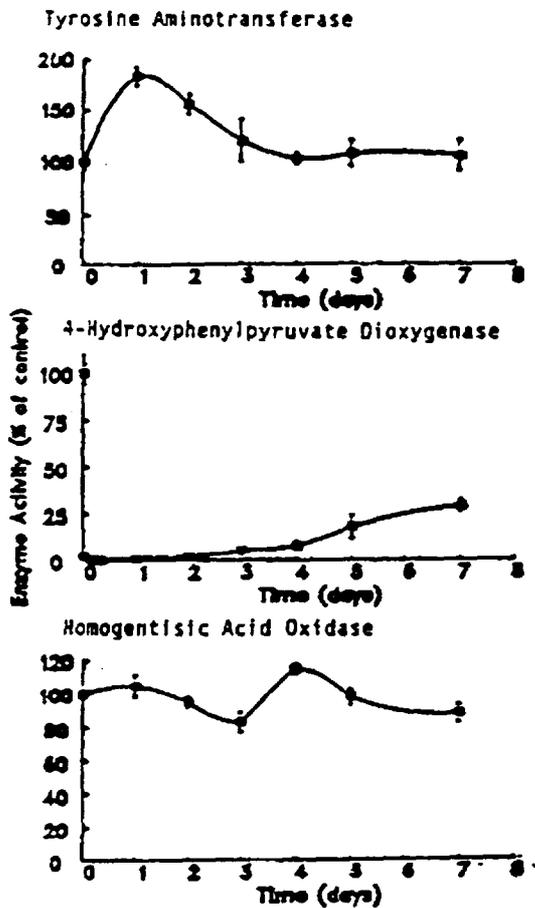


APPEARS THIS WAY  
ON ORIGINAL

# BEST POSSIBLE COPY

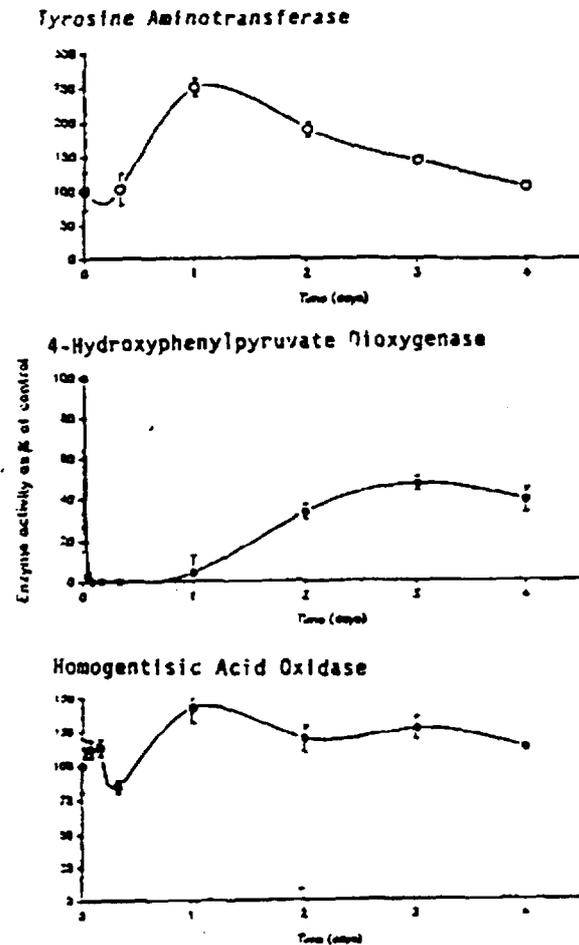
Sponsor's Figure Vol. 1.7/042

THE EFFECT OF A SINGLE ORAL DOSE OF SC-0735 AT 30 $\mu$ mol/kg ON THE ACTIVITY OF HEPATIC TYROSINE AMINOTRANSFERASE, 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE AND HOMOGENTISIC ACID OXIDASE AT VARIOUS TIMES AFTER DOSING.



Sponsor's Figure Vol. 1.7/043

THE EFFECT OF A SINGLE ORAL DOSE OF SC-0735 AT 0.3 $\mu$ mol/kg ON THE ACTIVITY OF HEPATIC TYROSINE AMINOTRANSFERASE, 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE AND HOMOGENTISIC ACID OXIDASE AT VARIOUS TIMES AFTER DOSING.



APPEARS THIS WAY  
ON ORIGINAL

# BEST POSSIBLE COPY

Sponsor's Table Vol. 1.7/044

THE DISTRIBUTION OF RADIOLABEL FROM SC-0735 IN RAT TISSUES AFTER A SINGLE ORAL DOSE OF 30 $\mu$ mol/kg BODYWEIGHT

Tissue	Tissue Concentration (nmol equiv/ml or g wet weight)								
	Time after dosing								
	1hr	2hr	4hr	8hr	24hr	2d	3d	4d	7d
Plasma	51 $\pm$ 16	108 $\pm$ 12	93 $\pm$ 5	69 $\pm$ 3	11 $\pm$ 1	1.13 $\pm$ 0.03	0.45 $\pm$ 0.02	0.20 $\pm$ 0.02	0.12 $\pm$ 0
Cornea	8.9 $\pm$ 0.4	11.6 $\pm$ 1.7	14.9 $\pm$ 0.7	9.3 $\pm$ 0.8	1.2 $\pm$ 0.1	0.16 $\pm$ 0	<0.05	<0.05	<0.05
Remainder of eye	7.1 $\pm$ 0.5	11.9 $\pm$ 1.6	10.4 $\pm$ 0.7	8.4 $\pm$ 0.5	1.3 $\pm$ 0.1	0.30 $\pm$ 0.02	<0.05	<0.05	<0.05
Extra-orbital lacrymal gland	15.0 $\pm$ 1.2	19.8 $\pm$ 2.0	17.3 $\pm$ 1.4	12.0 $\pm$ 0.6	2.0 $\pm$ 0.1	0.23 $\pm$ 0.01	0.32 $\pm$ 0.01	<0.05	<0.05
Intra-orbital lacrymal gland	-	29.2 $\pm$ 4.6	21.9 $\pm$ 1.4	15.5 $\pm$ 0.9	2.4 $\pm$ 0.2	0.28 $\pm$ 0.01	-	-	-
Harderian gland	16.2 $\pm$ 1.2	24.9 $\pm$ 2.8	24.0 $\pm$ 1.2	21.7 $\pm$ 0.8	13.2 $\pm$ 0.2	2.80 $\pm$ 0.22	2.20 $\pm$ 0.5	0.54 $\pm$ 0.06	0.15 $\pm$ 0.01
Liver	38.5 $\pm$ 3.1	61.6 $\pm$ 7.0	49.2 $\pm$ 1.8	37.5 $\pm$ 1.8	11.4 $\pm$ 0.4	6.58 $\pm$ 0.13	9.20 $\pm$ 0.5	6.70 $\pm$ 0.1	7.3 $\pm$ 0.6
Kidney	28.3 $\pm$ 1.3	34.1 $\pm$ 2.5	32.6 $\pm$ 2.3	24.3 $\pm$ 1.2	8.4 $\pm$ 0.3	2.54 $\pm$ 0.05	1.70 $\pm$ 0.08	1.7 $\pm$ 0.05	1.6 $\pm$ 0.06
Lung	17.7 $\pm$ 2.8	20.1 $\pm$ 2.4	19.2 $\pm$ 1.6	13.7 $\pm$ 1.0	2.1 $\pm$ 0.1	0.29 $\pm$ 0.01	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01	<0.05
Brain	2.1 $\pm$ 0.2	3.2 $\pm$ 0.5	3.0 $\pm$ 0.2	2.1 $\pm$ 0.2	0.3 $\pm$ 0.1	<0.05	<0.05	<0.05	<0.05

Values are mean  $\pm$  SE with four animals per time point, - not determined.

# BEST POSSIBLE COPY

Sponsor's Table Vol. 1.7/045

THE DISTRIBUTION OF RADIOLABEL FROM SC-0735 IN RAT TISSUES AFTER A SINGLE ORAL DOSE OF 0.3 $\mu$ mol/kg BODYWEIGHT

Tissue	Tissue Concentration (pmol equiv/ml or g wet weight)							
	Time after dosing							
	1hr	2hr	4hr	8hr	24hr	2d	3d	4d
Plasma	24.5 $\pm$ 2.3	70.5 $\pm$ 19.6	46.0 $\pm$ 8.3	97.3 $\pm$ 25.6	<1	<1	<1	<1
Cornea	<1	<1	<1	<1	<1	<1	<1	<1
Remainder of eye	4.6 $\pm$ 1.0	8.4 $\pm$ 2.0	11.8 $\pm$ 3.6	14.1 $\pm$ 4.0	<1	<1	<1	<1
Extra-orbital lacrymal gland	15.0 $\pm$ 1.6	22.7 $\pm$ 3.6	17.2 $\pm$ 3.3	27.0 $\pm$ 4.5	<1	<1	<1	<1
Harderian gland	26.5 $\pm$ 2.3	49.0 $\pm$ 11.2	67.0 $\pm$ 10.7	197.5 $\pm$ 27.0	85.5 $\pm$ 6.8	29.7 $\pm$ 3.2	8.3 $\pm$ 1.9	<1
Liver	2130 $\pm$ 214	2633 $\pm$ 201	3232 $\pm$ 124	3317 $\pm$ 117	3425 $\pm$ 123	2717 $\pm$ 59	2521 $\pm$ 322	2161 $\pm$ 284
Kidney	939 $\pm$ 77	947 $\pm$ 34	1055 $\pm$ 69	992 $\pm$ 44	967 $\pm$ 53	947 $\pm$ 36	964 $\pm$ 83	881 $\pm$ 24
Lung	17.4 $\pm$ 2.7	27.0 $\pm$ 6.7	21.5 $\pm$ 5.4	27.1 $\pm$ 5.7	<1	<1	<1	<1
Brain	<1	<1	<1	<1	<1	<1	<1	<1

Values are mean  $\pm$  SE with four animals per time point.

**Pharmacokinetic Evaluation and Bioavailability: NTBC Pharmacokinetics in the Rat.**

Report 1995-10-10.

Vol. 1.7/098

Statistics: Pitman randomization tests were used for the comparison of values from two independent populations. 6-7 rats per group. Intravenous dose: (?). Dose solution:  $3.03 \pm 0.041$ . Dose suspension:  $2.61 \pm 0.142$ .

Reviewer's Table: Mean Values

Dose	AUC	AUC norm. Coef. var. %	Bioavailability % (95%CI)	Tmax (hrs) Std. Dev. ( )	Cmax ( $\mu\text{g/ml}$ ) Std.Dev. ( )
Intravenous -	171.99	14	-	9.3	-
Oral Suspension $2.61 \pm 0.142 \text{ mg/kg}$	135.98	17	90.6 (71-112)	5.49 (2.63)	6.57 (1.77)
Oral Solution $3.03 \pm 0.041 \text{ mg/kg}$	180.18	8.4	101 (89-121)	3.57 (3.28)	10.10 (2.49)

There was no statistical difference in dose normalized AUC values from rats treated with:

Oral suspension and intravenous injection ( $p=0.25$ )

Oral solution and intravenous injection ( $p=0.77$ )

Oral solution and Oral suspension ( $p=0.11$ )

Neither was there any difference in time to reach maximum plasma concentration ( $p=0.27$ ).

**Human:**

A summary (Vol.1.1/164) was given for a single dose (1 mg/kg), cross-over, pharmacokinetic study of capsule and liquid formulations (composition not given) of NTBC in 10 healthy volunteers age 19-39 with a mean weight of 75.5 kg. It is reported that there were no statistical differences in AUC and  $T_{1/2}$  and that the two formulations were bioequivalent (values?).  $C_{\text{max}}$  for the liquid was 2 hours and 1.6 to 11.1 hours for the capsule. The coefficients of variation of AUC were 25.6 and 23.7% and  $C_{\text{max}}/\text{AUC}$  16.0 and 14.7% for the capsule and liquid formulations, respectively. Tyrosinemia was induced in all volunteers. This increased in a near linear fashion for 48 hrs. after dosing and then remained constant at ca. 1200 nmol/ml plasma for the experimental period of 120 hrs. The mean maximal tyrosine concentration induced was  $1155 \pm 121.2$  nmol/ml. After a 14 day washout period, mean plasma levels were still elevated at about  $808.4 \pm 222.8$  nmol/ml. After the second dose, tyrosinemia increased by about 300 nmol/ml within 48 hrs. to ca  $1162 \pm 251.2$  nmol/ml. Fasted follow-up samples from 7 subjects a few weeks after the study showed tyrosine levels back to normal.

**SC-0735 Metabolism Probe in Rats: Report Nos. T-13585 and 88-06-13**

dtd. 13 Jun 88. Summary Report Vol. 1.7/111.

One male and one female per group Sprague-Dawley rats (CrI:CD(SD)BRAVF/Plus - 7-9 wks. of age) were administered a single oral dose of 100 mg/kg (ca 1.0  $\mu\text{Ci/g}$ ) in corn oil of  $^{14}\text{C}$ -[Benzene Ring]-SC-0735 (Lot -10762-3-1 Specific activity 29.8 mCi/mmol - purity) or  $^{14}\text{C}$ -[Dione Ring]-SC-0735 (Lot -10687-42-4 Specific activity 20.2 mCi/mole - purity). Animals were sacrificed after 6 hours and urine, feces and selected tissues including the eyes, were saved and analyzed for radioactive content and metabolite patterns.

Overall recovery, male and female, averaged 87.3% after  $^{14}\text{C}$ -[Benzene Ring]-SC-0735 and 104.1% after  $^{14}\text{C}$ -[Dione Ring]-SC-0735 administration. Six hours after dosing only 3.3-6.7% of the radiolabeled dose had been excreted in the urine. There was little or no fecal excretion during this time period.

A preliminary observation showed higher concentrations of radioactivity in tear-forming tissues of the eye than in the vitreous humor or lens, suggesting that tears may be a major route or exposure

to the cornea. This preliminary assessment of metabolite patterns in the urine and eyes demonstrated that extensive metabolism of SC-0735 occurs, and that the metabolic pattern of the eye is much more complex than the pattern in the urine. In general there was no distinct difference based on sex or site of the  $^{14}\text{C}$  label observed in the concentration of radioactivity in ocular tissues.

**APPEARS THIS WAY  
ON ORIGINAL**

Sponsor's Tables Vol. 1.7/117-119

**Recovery of Radioactivity in Urine, Feces, Tissues,  
and Cage Wash 6 Hours After 100 mg/kg  $^{14}\text{C}$ -SC-0735**

Animal No.	283	263	284	264
Sex	Male	Female	Male	Female
Site of $^{14}\text{C}$ -Label	Benzene Ring	Benzene Ring	Dione Ring	Dione Ring
Percent of Radioactive Dose Recovered at 6 hours				
Urine	3.34	4.57	6.67	4.94
Feces	N.D. <sup>b</sup>	---	---	N.D.
Liver	6.58	7.57	6.83	7.92
Kidneys	0.87	0.94	1.07	1.20
Sciatic Nerve	0.01	0.01	0.01	0.02
Skin	11.38	12.23	11.88	14.43
Left Femur	0.08	0.09	0.04	0.12
Left Eye	0.16	0.21	0.16	0.31
Whole Blood	4.59	6.24	4.49	9.13
Organs <sup>c</sup>	31.66	24.19	39.87	22.46
Carcass	27.66	31.73	32.55	42.99
Cage Wash	0.24	0.49	0.29	0.79
<b>TOTAL</b>	<b>86.24</b>	<b>88.24</b>	<b>103.86</b>	<b>104.24</b>

<sup>a</sup> No Feces.

<sup>b</sup> N.D. = not determined.

<sup>c</sup> Organs = remaining internal organs, including small and large intestines, heart, lungs, spleen and mesentery.

**APPEARS THIS WAY  
ON ORIGINAL**

APPEARS THIS WAY  
ON ORIGINAL

The Concentration of Radioactivity in Tissues of Rats 6 Hours After 100 mg/kg <sup>14</sup>C-SC-0735

Tissue	Benzene Ring Label				Dione Ring Label			
	Male (#283)		Female (#263)		Male (#284)		Female (#264)	
	Concentration <sup>a</sup>	T/P <sup>b</sup>	Concentration	T/P	Concentration	T/P	Concentration	T/P
Plasma	160	---	197	---	191	---	262	---
Whole Blood	122	0.76	144	0.73	131	0.69	182	0.69
Liver	149	0.93	157	0.80	154	0.81	188	0.72
Kidneys	75	0.47	89	0.45	90	0.47	112	0.43
Sciatic Nerve	59	0.37	63	0.32	67	0.35	75	0.29
Skin	48	0.30	63	0.32	52	0.27	73	0.28
Left Femur	26	0.16	25	0.13	19	0.10	29	0.11
Organs <sup>c</sup>	257	1.60	240	1.22	290	1.52	221	0.84
Carcass	40	0.25	41	0.21	49	0.26	56	0.21
Left Eye Globe <sup>d</sup>	61	0.38	66	0.34	74	0.39	93	0.35
Left Eye Secretory Tissues <sup>e</sup>	73	0.46	55	0.28	75	0.39	104	0.40

- <sup>a</sup> Concentration = ug equivalents/g tissue.
- <sup>b</sup> T/P = ratio of tissue to plasma concentration.
- <sup>c</sup> Organs = remaining internal organs, including small and large intestines, heart, lungs, spleen, and mesentery.
- <sup>d</sup> Left eye globe = cornea, corneal epithelium, lens, aqueous and vitreous humors, and the residuum.
- <sup>e</sup> Left eye secretory tissues = lachrymal glands, Harderian gland and eyelids.

APPEARS THIS WAY  
ON ORIGINAL

# BEST POSSIBLE COPY

NDA 21-232 p.14

The Concentration of Radioactivity In Eye Tissues 6 Hours After 100 mg/kg <sup>14</sup>C-SC-0735

Tissues	ug Equivalents SC-0735/g Tissue						
	<sup>14</sup> C-[Benzene Ring] Label			<sup>14</sup> C-[Dione Ring] Label			Ratio (Males) <sup>a</sup>
	Male (#283)	Female (#263)	Ratio (Male/Female)	Male (#284)	Female (#264)	Ratio (Male/Female)	[Dione/Benzene]
Cornea	73	78	0.94	97	119	0.82	1.33
Lens	17	19	0.90	16	21	0.79	0.95
Extraorbital lacrimal gland	64	36	1.81	69	90	0.76	1.07
Harderian gland	84	90	0.93	82	123	0.66	0.97
Eyelids	61	66	0.93	68	92	0.74	1.11
Residuum	70	78	0.90	81	105	0.77	1.15
Vitreous Humor	33	41	0.81	47	64	0.74	1.42
Corneal Epithelium	73	105	0.70	111	132	0.84	1.51
Plasma	160	197	0.81	191	262	0.73	1.19

<sup>a</sup> Ratio (males) = (Tissue concentration for animal #284) ÷ (Tissue concentration for animal #283)

APPEARS THIS WAY  
ON ORIGINAL

**SINGLE DOSE TOXICITY STUDIES****Acute Toxicity:** [Vol. 1.5/25 No data]

Reported Oral: Mortality

- Rats – male and female - 0/10 at 100 mg/kg
- Females - 8/10 at 1000 mg/kg

Mice – LD<sub>50</sub> 637 mg/kg in males and 796 mg/kg in females**REPEATED DOSE TOXICITY STUDIES**

Additional Comments re Study T-12984 reference IND review attached, p. 6.

T-12984 Integrated 13 week Subchronic and 2 Year Chronic (12 mo.) Toxicity/Oncogenicity Study with SC-0735 in Rats. Compiled by \_\_\_\_\_ Report 1996-05-08.

Summary: Interruption of study:

Almost a year after the start of the integrated toxicity/carcinogenicity study it was decided to terminate the study earlier than originally scheduled because development plans for the original intended use were cancelled. The study design was revised in order to collect data, which were considered valuable for future research. The originally scheduled 12-month interim necropsy was conducted and tissues and data were stored but not compiled and evaluated.

When it was found that SC-0735 was a potent inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase and had a potential therapeutic effect in patients suffering from hereditary tyrosinemia type 1 there was a renewed need to evaluate the toxicity of SC-0735. Therefore, it was decided to evaluate the remains of the study in rats. Thus, the sponsor concluded that the only way to make use of the data of the study was to summarize the major findings in a scientific format. The study was reported as conducted according to GLP, but there was no QA. A selected list of appropriate tissues from the 12 month interim necropsy were examined histopathologically (Report — T/2906)

**Methods and material:**

The original purpose of the study was to determine the 3-month subchronic toxicity and the 2 year chronic toxicity/oncogenicity of SC-0735 administered in the diet at 0, 1, 5, 40, 120, 320 and 800 ppm (ca 0.05, 0.25, 2, 6, 16 and 40 mg/kg) to 70 rats per group and sex. Ten animals were removed from each dose group for a 3-month necropsy. Based on analysis from those rats all remaining rats from the 5 and 120 ppm dose groups were considered surplus and sacrificed.

When plans for development of SC-0735 were cancelled it was decided to modify the study and only perform the scheduled necropsy after 12 months on 10 animals of each sex of the remaining groups and sacrifice the rest of the treated animals without necropsy except for a few to be used for an ocular lesion recovery study. (Report T-13249 - 88-06-27 IND Review p. 6 indicated little or no ocular lesion recovery).

**Results:**

Interim Sacrifice after 3 Months: [Reported by \_\_\_\_\_ (87-07-31) no report has been found from the interim sacrifice at 3 months.] Summary Only (raw data 4, 6 13 months appended as Report T-12984): [See Reviewer's tables below.]

Ocular changes appeared in all treatment groups – about 50 % of the males and 25% of the females. The principal manifestation of toxicity was a dose-related growth depression. Statistically significant increases were seen in organ weights in relation to body weights for kidneys and livers of all dosed males and kidneys and livers of all dosed females except the lowest dose group. None of the absolute organ weights or organ weights in relation to brain weights were significantly increased and there were no treatment-related histopathological changes in these organs at the highest dose level, 800 ppm. There were a number of statistically significant differences in the clinical chemistry and hematology between the treated groups and controls after 3 months but most of them did not remain after 12 months.

# BEST POSSIBLE COPY

NDA 21-232 p.16

Final Sacrifice after 12 Months: — Summary Only (raw data 4, 6, 13 months appended as Report T-12984) [See Tables below.]

Besides the observed corneal opacities there were no clinical observations reported which were considered of importance.

There was a dose-related decrease in body weight gain particularly above 40 ppm in both males and females concomitant with a slight decrease in food consumption.

There was a slight but statistically significant decrease in males of the two high dose groups in hematocrit, hemoglobin, and number of red blood cells. There was also a slight increase in mean corpuscular volume and mean corpuscular hemoglobin. Females showed only a slight increase in mean corpuscular hemoglobin.

There was a marked and statistically significant decrease in blood glucose concentration in males and females of the highest dose. There was a slight increase in serum potassium in females of the two high dose groups.

Necropsy showed a statistically significant increase in kidney weight in both absolute and relative body and relative brain weight in the three high dose (40, 320, 800 ppm) male groups. Liver weight was increased relative to brain weight in the two low dose groups (1 and 40 ppm). Females showed no similar organ weight increases.

Macroscopically there were no remarkable findings.

Histopathological examination of selected tissues showed a dose related corneal keratitis in all dose groups. There was an increase slight centrilobular hypertrophy in the livers of 800 ppm females. Other histopathological findings were not considered treatment related.

The overall incidence of tumors was reported as low and not treatment related.

## Note:

Eyes were affected showing a dose related corneal keratitis at 1 ppm (0.05 mg/kg) and above.

In general while organ weight increases were reported mainly in males, histopathological changes observed were found mainly in females.

Due to the reduced number of animals and the reduced time of exposure the response to SC-0735 carries little or no weight as a carcinogenicity study.

Report — /P/4710 ( — Study PR1010) Gives: Histopathological Examination of Selected Tissues - Individual Pathology Data Supplement (print ok) and Appendix to Report T-12984 gives Raw data Listings for body weight, food consumption, clinical observations, chemistry, hematology (all Poor quality print - difficult to read).

Differences from control included:

Sponsor's Table Vol.1.5/215

Incidences of Ocular Lesions in the  
SC-0735 Integrated Rat Study

Time (Weeks)	SC-0735 (ppm)	Ocular lesion incidence in	
		Males	Females
4-8	0 (control)	0/70	0/70
	1	17/70	1/70
	5	31/70	15/70
	40	31/70	12/70
	120	34/70	19/70
	320	33/68	22/70
	800	21/69	16/68

Incidences were higher in males than in females and they increased in both sexes between 4 and 8 weeks.

Mainly significant differences from controls (other than sporadic - as close as could be ascertained from tables – poor print in general included the following: [Reviewer's Tables]

**Hematology:** [Reviewer's Tables]

Doses 3,4 months 0, 1, 5, 40, 120, 320 800  
 6,12,13 months 0, 1, -, 40, - , 320, 800 ppm

**Increases**

	Months	Dose Level (ppm)
<b>Increases Males</b>		
Mean Corpuscular Volume	3	5,40,120,320,800
	6	320,800
	12,13	40,320,800
Mean Corpuscular Hb (per g)	3,4	120, 320, 800
	6	320,800
	12, 13	40,320,800
<b>Increases Females</b>		
Mean Corpuscular Volume	3	40,120
Mean Corpuscular Hb (per g)	3	1,5,40,120
	4	40,120
	6	40
	12	1,40,320
Potassium	13	320,800

**Decreases**

	Months	Dose Level (ppm)
<b>Decreases Males</b>		
RBC's	3	40,120,320,800
	4	800
	6	320,800
	12,13	40,320,,800
Hemoglobin	3	800
	6	320
	12	320,800
Hematocrit	3	120,320,800
	12	320,800
Platelets	4	120,320,800
<b>Decreases Females</b>		
RBC's	3	1,40,800
Platelets	3	1,5,40120,320,800
	12	1,320
Lymphocyte (%)	6,12	800
Sodium	6	1,40
	13	40

**Clinical Chemistry: [Reviewer's Tables]**

Doses 4 Months 0, 1, 5, 40, 120, 320, 800 ppm

6, 13 Months 0, 1, -, 40, -, 320, 800 ppm

**Increases:**

	Months	Dose Level (ppm)
<b>Increases Males:</b>		
Calcium	4,13	1-320
Phosphorus	4	5-120
Albumin	4	40, 320, 800
A/G Ratio	4	800
Brain cholinesterase	4	5-800
<b>Increases Females:</b>		
Phosphorus	4	5-320
Sorbitol dehydrogenase	6	40,800
Brain Cholinesterase	4	5,120, 320
	13	800

**Decreases:**

	Months	Dose Level (ppm)
<b>Decreases Males</b>		
Glucose	4,6,13	800
	6	320,800
Creatinine Phosphokinase	4	40-320
<b>Decreases Females</b>		
Glucose	4	5,320,800
	6	320,800
	13	800
Creatinine	6	1,40,320,800
	13	1,40,320
Serum Cholinesterase	6	320,800

**APPEARS THIS WAY  
ON ORIGINAL**

### Table for Body and Organ Weights

Adapted from Sponsor's Tables Vol.1.5 (Poor Quality and markings due to Sponsor's Tables.)

STUDY:12984M SC-0735 INTEGRATED SUBCHRONIC/CHRONIC IN RATS  
ORGAN WEIGHTS MEANS BY GROUP

ORGAN NAME	DOSE GROUP	--ABSOLUTE WEIGHT--				--% OF BODY WEIGHT--				--% OF BRAIN WEIGHT--			
		MEAN	* CTL	N	STD. DEV.	MEAN	* CTL	N	STD. DEV.	MEAN	* CTL	N	STD. DEV.
<b>Males Sacrifice 3 months Vol. 1.5/244</b>													
KIDNEYS	10	3.409	100	10	0.398	0.594	100	9	0.044	161.915	100	10	22.293
	20	3.693	108	10	0.302	0.687b	117	10	0.038	172.206	106	10	13.647
	30	3.624	106	10	0.489	0.700b	117	10	0.073	174.388	107	10	19.847
	40	3.635	106	10	0.265	0.711b	119	10	0.063	172.254	106	10	12.304
	50	3.717	109	10	0.315	0.732b	123	10	0.061	180.094	111	10	18.830
	60	3.593	105	10	0.324	0.775b	130	10	0.072	177.443	109	10	16.848
	70	3.542	103	10	0.325	0.784b	131	10	0.052	174.043	107	10	11.796
LIVER	10	15.543	100	10	2.489	2.769b	100	9	0.222	759.408	100	10	137.659
	20	17.536	112	10	2.877	3.220b	119	10	0.283	817.324	110	10	127.721
	30	16.520	106	10	2.340	3.183b	117	10	0.254	795.263	107	10	98.444
	40	16.466	105	10	1.710	3.207b	118	10	0.213	780.567	105	10	83.864
	50	15.596	100	10	1.611	3.072b	113	10	0.295	754.479	102	10	77.496
	60	15.014	96	10	1.767	3.226b	119	10	0.255	740.866	100	10	94.559
	70	14.783	95	10	1.277	3.270b	121	10	0.181	726.625	98	10	46.665

### Females Sacrifice 3 months Vol. 1.5/249

KIDNEYS	15	1.754	100	10	0.171	0.777b	100	10	0.061	76.000	100	10	9.175
	25	1.780	103	10	0.207	1.255b	105	10	0.069	105.112	107	10	10.919
	35	1.717	103	10	0.155	1.705b	113	9	0.055	99.910	104	10	9.059
	45	1.892	107	10	0.155	3.701b	113	10	0.052	100.721	104	10	9.431
	55	1.764	105	10	0.155	0.701b	113	10	0.056	104.391	108	10	10.557
	65	1.752	94	10	0.255	0.717b	113	10	0.057	95.252	97	10	4.246
	75	1.897	91	10	0.194	0.751b	114	10	0.071	92.843	93	10	6.251
LIVER	15	7.500	100	10	0.171	2.514	100	10	0.251	390.055	100	10	47.999
	25	7.716	105	10	1.235	2.921	106	10	0.275	410.292	106	10	65.767
	35	7.576	101	10	0.924	2.771	113	9	0.220	374.440	101	10	53.059
	45	7.752	105	10	0.421	2.956b	114	10	0.217	410.049	105	10	20.310
	55	7.587	104	10	0.778	2.335b	112	10	0.275	431.930	108	10	48.376
	65	7.115	93	10	1.130	3.922b	117	10	0.302	372.785	97	10	24.642
	75	7.553	93	10	0.454	3.255b	120	10	0.244	391.564	100	10	32.959

### Males Sacrifice 12 months Vol. 1.5/258

KIDNEYS	10	5.752	100	10	0.522	0.477	100	10	0.047	164.225	100	10	15.375
	20	6.375	114	10	0.415	0.567	115	10	0.067	197.801b	120	10	13.950
	40	6.577	120	10	0.760	0.649b	133	10	0.111	215.405b	129	10	14.505
	60	6.017b	121	10	0.273	0.672b	137	10	0.097	213.755b	130	10	17.525
	70	6.437b	117	10	0.526	0.723b	149	10	0.085	217.121b	124	10	16.034
LIVER	10	17.443	100	10	2.065	2.490	100	10	0.267	544.272	100	10	119.546
	20	18.303	113	10	3.579	2.909b	120	10	0.279	1052.596b	124	10	151.038
	40	22.145	113	10	3.281	3.101b	124	10	0.350	1029.051b	121	10	146.033
	60	20.772	106	10	2.736	2.595b	119	10	0.309	960.099	118	10	175.111
	70	20.217	104	10	2.067	3.500b	132	10	0.397	944.064	111	10	155.443

### Females Sacrifice 12 months Vol. 1.5/264

KIDNEYS	15	2.310	100	10	0.211	0.550	100	10	0.094	111.935	100	10	8.027
	25	2.303	103	10	0.253	0.597	102	10	0.092	115.979	107	10	10.825
	45	2.315	100	10	0.199	0.572	104	10	0.094	116.497	104	10	12.046
	65	2.172	94	10	0.254	0.644	117	10	0.071	108.604	97	10	9.952
	75	2.054b	83	10	0.155	0.785b	129	10	0.100	105.040	93	10	7.770
LIVER	15	13.660	100	10	1.442	2.522	100	10	0.310	517.410	100	10	73.579
	25	10.340	96	10	1.379	2.574	102	10	0.253	515.476	100	10	75.721
	45	10.777	101	10	1.595	2.631	104	10	0.352	542.331	104	10	85.534
	65	9.452	88	10	1.566	2.794	110	10	0.277	471.529	91	10	44.538
	75	8.997b	84	10	1.056	3.047b	121	10	0.354	450.255	49	10	55.450

**REPRODUCTIVE TOXICOLOGY****Study title:** A Rangefinding Teratology Probe in CD Rats with SC-0735 Technical**Study No. Vol. and page number:** T-12935, Project 220567 dtd. 19 Jul 88. Vol. 1.6 p. 052**Site and testing facility:** \_\_\_\_\_**GRP compliance:** No given.**QA- Reports Yes ( ) No (X):****Lot:** EHC-0751-29**Methods:****Species/strain:** CD, Sprague-Dawley derived time-mated female rats**Route of Administration:** Oral by gavage for 13 consecutive days, gravid days 8-20.**Study Design:**

Ten time-mated female rats per group were dosed orally by gavage with 0, 20, 50, 125 mg/kg SC-0735 Technical in corn oil (reported to have adequate stability for 6 weeks) for 13 consecutive days, gravid days 8-20. [Suspension volume 5 ml/kg.]

Body weights (gravid days 7, 8, 9, 12, 16, 20), food consumption (gravid days 8-12, 12-16, 16-20 and postpartum days 0-4) and clinical observations were recorded. Dams and litters were examined at delivery and postpartum day 4 for the following: dam weight, total litter size, number of live and dead pups, gross external anomalies and total litter weight. The reproductive tract of all females was examined at necropsy (postpartum day 4 or 5), pregnancy status was determined and liver and kidney weights were determined.

**Comments:**

The purpose of this study was to determine dose levels for a definitive teratology study in rats with SC-0735 Technical and to provide an early assessment of both embryofetal toxicity and teratogenic effects.

Dose levels of 20, 50 and 125 mg/kg showed maternal toxicity. There were no statistically significant treatment related effects on mortality (one high dose found dead gravid day 19 was pregnant) or fertility. One resorbed litter was evident at 20 mg/kg. At 125 mg/kg there was a statistically significant treatment-related increase in chromorrhoea. Although not statistically significant, rough hair coat was seen in 5/10 high dose females.

Body weight was significantly reduced at 125 mg/kg and body weight gains were significantly reduced at 50 and 125 mg/kg.

Food consumption was significantly reduced at various intervals at 20, 50 mg/kg and all time intervals at 125 mg/kg.

No treatment-related findings were reported for necropsy. However, Body weight showed a statistically significant decrease. Relative (but not absolute) mean kidney weight increases were statistically significant at 125 mg/kg.

Embryofetal toxicity was noted at the two higher doses which included a reduction in the live number of pups per litter and litter weight at 50 and 125 mg/kg. In addition the mean pup weight, mean change in pup weight for postpartum days 0-4 and percent of pup viability were also significantly reduced at 125 mg/kg. The number of dead pups per litter was also significantly increased at the high dose. Most pup deaths were on postpartum day 0 or 1. At 125 mg/kg one-third of pup deaths were stillborn.

There was no evidence of teratogenicity at any dose level.

The sponsor recommended the high dose in a definitive rat teratology study to be less than 125 mg/kg.

The no effect level for embryofetal toxicity was reported to be 20 mg/kg.

See Summary of Litter Effects Table below:  
Sponsor's Table Vol. 1.6/066

	SC-0735 (mg/kg/day)							
	0		20		50		125	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Number of Litters	10		7 <sup>a</sup>		9		7	
Duration of Gestation (days)	21.4	0.5	21.6	0.5	22.3*	0.9	21.9	1.1
Total Born	11.6	2.3	11.9	3.5	9.3	3.8	9.4	3.2
Total Implants	12.6	2.7	12.7	2.3	11.3	3.0	10.7	3.4
Postimplantation Loss (%) <sup>b</sup>	8.1	7.6	7.9	17.0	21.2	23.5	24.1	32.1
Postpartum Day 0								
Number of Live Pups/Litter	11.4	2.1	11.7	3.4	9.0	3.5	6.4*	4.6
Number of Dead Pups/Litter	0.2	0.4	0.1	0.4	0.3	0.7	3.0	3.9
Percent Stillborn Pups	0.7	2.1	0.0	0.0	1.6	4.8	21.1	35.5
Litter Weight (g)	70.1	11.5	73.1	18.9	54.5	19.2	37.5*	21.6
Mean Pup Weight (g)	6.2	0.5	6.3	0.4	6.2	0.5	5.0*	0.8
Postpartum Day 4								
Number of Live Pups/Litter	11.2	2.5	10.6	2.8	7.9*	2.7	4.0*	2.8
Litter Weight (g)	109.2	21.5	98.3	23.4	72.3*	23.6	31.4*	29.8
Mean Pup Weight (g)	9.9	1.2	9.4	0.8	9.6	1.0	7.0*	2.5
Postpartum Days 0-4								
Mean Pup Weight Change (g)	3.7	1.0	3.1	0.6	3.4	0.7	1.5*	1.8
Percent Pup Viability <sup>c</sup>	96.7	8.0	90.6	7.8	89.6	13.4	11.9*	24.9

\* Significantly different from 0 mg/kg/day dose level, p < 0.05.  
<sup>a</sup> Female #170 (totally resorbed litter) not included.  
<sup>b</sup> Percent Postimplantation Loss = 100 - [(Viable Implants/Total Implants) x 100].  
<sup>c</sup> Percent Pup Viability represents the percent of liveborn pups surviving on postpartum day 4.

Study title: A Cross-Fostering Reproduction Probe in CD Rats with SC-0735

Study No: Vol. and page number: Summary Report for T-13247 dtd. 9 Mar 88 Vol. 1.6 p. 081

Site and testing facility: \_\_\_\_\_

GRP compliance: Not given.

QA- Reports Yes ( ) No (X): Reported that: Although the in-life portion of this study was monitored by the Quality Assurance Unit, neither the raw data nor the contents of this summary memorandum have been audited. No other final report will be issued for this study.

Lot and batch numbers: EHC-0866-19 Source Lot ERC-9421-10-2; Purity \_\_\_\_\_

Protocol reviewed by Division Yes ( ) No (X):

**Comments:** It is reported that the purpose of this probe was to assess the effects of intrauterine or lactational exposure to SC-0735 (Lot EHC-0866-19) on pup survival and the occurrence of eye lesions in pups.

**Methods:**

- **Species/strain:** CRL: CD(BR) rats.
- **Doses employed:** 0 or 100 mg/kg/day SC-0735
- **Route of Administration:** Oral
- **Study Design:** Groups of forty time mated CRL: CD (BR) female rats were treated by oral gavage with 0 or 100 mg/kg/day SC-0735 as a suspension in corn oil, (5 ml/kg gravid day eight body weight), for  $35 \pm 1$  days, gestational day 8 through postpartum day 20, (dams were not dosed for 24 hours after delivery). It is reported that concentration and homogeneity of dosing suspensions were verified. Body weights and food consumption were measured at various intervals and clinical observations were recorded. Dams and litters were examined as soon as possible after delivery and the following recorded: dam body weight, food consumption, total litter size, number of live pups, number of dead pups, gross external anomalies, and total litter weight.

Within 24 hours of delivery litters from 14 control dams and 14 treated dams were exchanged for cross-fostering. The remaining litters (15 control and 15 treated) were not cross-fostered. Cross fostering resulted in the following phases:

- no exposure during gestation, no exposure during lactation
- no exposure during gestation, SC-0735 exposure during lactation (fostered)
- SC-0735 exposure during gestation, no exposure during lactation (fostered)
- SC-0735 exposure during gestation, SC-0735 exposure during lactation.

Dams that delivered were sacrificed on postpartum days 21-23. Liver and kidneys were weighed and the reproductive tract examined. Eyes from treated and selected controls were collected (tissues not analyzed).

Postpartum Day 21: Pups weaned and placed on powdered control diet until an assessment of eye lesions was made (ca. one week). Pups reared by control dams were placed on diets containing 0 or 80 ppm SC-0735 resulting in the following groups:

- No exposure during gestation, no exposure during postweaning
- No exposure during gestation, SC-0735 exposure during postweaning
- SC-0735 exposure during gestation, no exposure during postweaning
- SC-0735 exposure during gestation, SC-0735 exposure during postweaning

Body weight, food consumption, clinical observations and results of ophthalmic examinations were recorded weekly for 8 weeks following which animals were sacrificed and selected eyes were collected for evaluation (not evaluated!).

- **Number of animals/sex/dosing group:** Forty females per group
- **Parameters and endpoints evaluated:** See study design above.
- **Statistical evaluations:**
  - Dose group data were analyzed by Fisher exact probability with Bonferroni's correction.
  - Litter data were analyzed by Mann-Whitney U rank test or Newman-Keuls test.
  - Quantitative or continuous data were analyzed by Dunnett or Newman-Keuls tests.

**Results:**

- **Clinical signs:** Significantly increase in cloudy eyes.
- **Mortality:** Reported no effect. 4/40 females in each group died prior to delivery and 29 litters were delivered in each of the control and treated groups.
- **Body weight:**
  - Maternal:
    - Gravid Days 16 and 20: significantly reduced. [mean body weight 260 vs. 285 and 303 vs. 340 g]
    - Body Weight Gain significantly reduced gravid day intervals 8-9, 9-12, 12-16 and 16-20.
      - [mean body weight changes 5.8, 5.2, 18, 43 vs. 9.5, 21, 31, 57 g]

Mean body weight postpartum day 0 for treated dams was significantly reduced compared to controls [248  $\pm$  23.4 vs. 270  $\pm$  33.8].

Mean body weights of treated dams were significantly reduced postpartum day 4-14 compared to untreated – this was regardless of whether they were rearing pups that had been exposed to drug during gestation or not. By postpartum day 21 bodyweights were not significantly different from controls.

**- Food consumption:**

Maternal:

Significantly reduced gravid day intervals 8-12, 12-16, 16-20 [13, 15, 20 vs. 21, 22, 27 g].

Significantly reduced during lactation for dams treated with SC-0735 and for untreated dams rearing pups exposed to SC-0735 during gestation compared to untreated dams rearing pups with no SC-0735 exposure.

**- Fertility and Early Embryonic Development in Females**

No statistically significant effects were reported for SC-0735 treatment on duration of gestation, percent of postimplantation loss or total number of pups per litter on postpartum day 0.

Although not statistically significant the percent live born pups and the number of live pups per litter on day 0 were reduced for treated dams compared to controls.

At birth mean pup weights of 100 mg/kg/day treated were significantly reduced [5.3  $\pm$  0.6 vs. 6.6  $\pm$  0.6].

A significantly greater number of treated pups had cloudy eyes than did controls.

**- Offspring:**

Pup Survival:

Postpartum Day 0:

No statistically significant differences in number of live pups on postpartum day 0 after cross-fostering.

Postpartum Day 4:

Number of live pups per litter and survival index were significantly reduced for litters exposed to SC-0735 during gestation compared to those with no prenatal treatment exposure, regardless of whether they were reared by treated or untreated dams.

After postpartum day 4, most pups survived.

Pup Weights:

Significantly reduced for pups exposed to SC-0735 during gestation on Postpartum day 0 after cross-fostering and on Postpartum day 4 compared to pups without prenatal compound exposure whether or not reared by treated or untreated dams.

Postpartum day 7 mean pup weights were significantly reduced for all pups with any exposure to SC-0735 compared to pups with no compound exposure. Pups exposed both during gestation and lactation weighed significantly less than pups exposed only during a single period. Weights were still reduced in a similar manner on Postpartum day 14. By day 21 pups exposed during gestation and lactation compared to a single exposure period had recovered, however, each of these groups weighed significantly less than that for pups with no exposure to SC-0735.

Eye Opening:

The mean day postcoitus on which 50% of each litter had open eyes was significantly delayed for pups exposed to treatment only during gestation or during gestation and lactation.

Pup Morphological Examination:

Performed on pups found dead shortly after birth. In pups exposed to treatment during gestation there was an increase in the incidence of urinary tract findings including the presence of white material in the urinary tract. The urinary tracts of weanlings of born to control dams and reared by either control or treated did not show such findings.

Weanling Ophthalmological Examinations:

Corneal opacities were observed in nearly all pups reared by treated dams. Lesions in pups with no compound exposure during gestation which were reared by treated dams (lactation exposure only) were more severe than for pups exposed to drug during gestation which were reared by treated dams (gestational and lactational exposure). Gestational exposure alone did not increase the incidence of corneal lesions in weanlings.

**APPEARS THIS WAY  
ON ORIGINAL**

BEST POSSIBLE COPY

SUMMARY OF LITTER EFFECTS (POST-CROSS-FOSTERING)				
LACTATIONAL EXPOSURE	DOSE LEVEL (MG/KG/DAY)			
	0		100	
GESTATIONAL EXPOSURE	0	100	0	100
<u>LITTERS WITH PUPS</u>				
POSTPARTUM DAY 0	15	14	14	15
POSTPARTUM DAY 4	15	8	14	6
<u>MEAN PUP WEIGHT (GRAMS)</u>				
POSTPARTUM DAY 0	6.6 <sup>a</sup>	5.4 <sup>b</sup>	6.8 <sup>a</sup>	5.2 <sup>b</sup>
POSTPARTUM DAY 4	10.6 <sup>a</sup>	7.5 <sup>b</sup>	9.4 <sup>a</sup>	6.8 <sup>b</sup>
POSTPARTUM DAY 7	15.5 <sup>a</sup>	12.0 <sup>b</sup>	12.6 <sup>b</sup>	9.2 <sup>c</sup>
POSTPARTUM DAY 14	29.0 <sup>a</sup>	25.2 <sup>a,b</sup>	24.5 <sup>a,b</sup>	19.3 <sup>c</sup>
POSTPARTUM DAY 21	45.9 <sup>a</sup>	38.9 <sup>b</sup>	38.7 <sup>a,b</sup>	32.5 <sup>b</sup>
<u>VIABILITY (%)</u>				
PUPS LIVE TO DAY 4 (SURVIVAL)	99 <sup>a</sup>	33 <sup>b</sup>	99 <sup>a</sup>	28 <sup>b</sup>
LACTATION (4-21)	99 <sup>a</sup>	85 <sup>a</sup>	100 <sup>a</sup>	81 <sup>a</sup>
PUPS LIVE TO DAY 21	98 <sup>a</sup>	31 <sup>b</sup>	99 <sup>a</sup>	25 <sup>b</sup>
<u>MEAN NUMBER LIVE PUPS/LITTER</u>				
POSTPARTUM DAY 0	10.9 <sup>a</sup>	10.3 <sup>a</sup>	10.3 <sup>a</sup>	8.8 <sup>a</sup>
POSTPARTUM DAY 4	10.8 <sup>a</sup>	6.5 <sup>b</sup>	10.2 <sup>a</sup>	6.8 <sup>b</sup>
POSTPARTUM DAY 7	10.8 <sup>a</sup>	7.1 <sup>b</sup>	10.2 <sup>a</sup>	6.5 <sup>b</sup>
POSTPARTUM DAY 14	10.8 <sup>a</sup>	7.0 <sup>b</sup>	10.2 <sup>a</sup>	6.3 <sup>b</sup>
POSTPARTUM DAY 21	10.7 <sup>a</sup>	7.0 <sup>b</sup>	10.1 <sup>a</sup>	7.4 <sup>a</sup>
<u>EYE OPENING</u>				
MEAN DAY 50% LITTER OPEN				
POSTPARTUM	14.5 <sup>a</sup>	15.3 <sup>a</sup>	14.9 <sup>a</sup>	16.2 <sup>b</sup>
POSTCOITUS	35.8 <sup>a</sup>	36.9 <sup>b</sup>	36.4 <sup>a,b</sup>	38.2 <sup>c</sup>
% OPEN POSTPARTUM DAY 15	90 <sup>a</sup>	59 <sup>b</sup>	74 <sup>a,b</sup>	20 <sup>c</sup>

values sharing the same superscript (a-c) in the same row are not significantly different (p > 0.05).

APPEARS THIS WAY  
ON ORIGINAL

**Dams after Weaning:**

**Ophthalmologic Exams:**

SC-0735 significantly increased the incidence of corneal opacities.

**Necropsy Findings and Organ Weights:**

Other than the corneal opacities there were no significant treatment-related necropsy findings other than increased liver and kidney weights relative to body weight (but not absolute) in the treated group.

**Post Weaning Pups:**

Pups reared by control dams were given diets of 0 or 80 ppm SC-0735 for 8 wks. to determine how rapidly eye lesions develop in young animals and whether prenatal exposure to the compound alters that development.

**Ophthalmologic Exams:** (weekly during the 8 wk. Postweaning phase)

After only 2 wks. of dietary exposure to 80 ppm SC-0735, 30% or more had corneal lesions, regardless of whether or not they were exposed to SC-0735 prenatally.

Sporadic findings were seen in animals that were prenatally exposed to compound and fed 0 ppm during the postweaning phase.

**Lens lesions:** Were observed mainly in animals with prior prenatal treatment exposure, regardless of whether they were fed 0 or 80 ppm SC-0735 during the post weaning phase.

**Body Weight, Body Weight Gain and Food Consumption:**

**Males:**

**Prenatally exposed:** These parameters were significantly reduced compared to no prenatal exposure regardless of the postweaning phase.

Further exposure to 80 ppm SC-075 in the diet did not result in sig. reductions in body wt. or food consumption. Body weight gain was only sporadically reduced (wks. 3 and 8) compared to exposure to 0 ppm diet and prenatally-exposed.

**No Prenatal Exposure:** Exposure to 80 ppm SC in diet significantly reduced body weight after two weeks, which persisted throughout the 8 wk period.

Body weight gain was significantly reduced during the first 2 wks. with a non-significant reduction except for week 8.

Food consumption was significantly reduced after 7 wks. of SC-075 in the diet.

**Females:**

**Prenatally exposed:** Body weight was significantly reduced compared to Females with no prenatal exposure regardless of postweaning exposure.

Body weight gain and food consumption were significantly reduced regardless of postweaning exposure relative to no compound exposure.

Exposure of SC-0735 in the diet did not cause significant reductions in body weight compared to prenatally exposed that received no drug in the diet.

**No Prenatal Exposure:** SC-0735 exposure during the postweaning phase caused no significant reduction in body weight except for the third week of study.

**APPEARS THIS WAY  
ON ORIGINAL**

Sponsor's Table Vol. 1.6/125

T-13247: SC-0735 CROSS-FOSTERING REPRODUCTION PROBE IN RATS				
SUMMARY OF WEANLING OPHTHALMIC EXAMINATIONS				
<u>LACTATIONAL EXPOSURE</u>	DOSE LEVEL (MG/KG/DAY)			
	<u>0</u>	<u>100</u>	<u>0</u>	<u>100</u>
<u>GESTATIONAL EXPOSURE</u>	<u>0</u>	<u>100</u>	<u>0</u>	<u>100</u>
<u>PUPS EXAMINED</u>	83	44	81	37
<u>CORNEAL OPACITIES</u>				
ROUGH (HAZY)	0	0	4	2
FOCAL	1	0	51	29
FOCAL WITH VASCULARIZATION	0	1	26	6
<u>OTHER</u>				
PERSISTENT PUPILLARY VESSELS	0	4	9	2
CONGESTED CIRCUMCORNEAL VESSELS	0	4	2	0
<u>LENS OPACITIES</u>	1	1	5	3

No statistical analyses were conducted on these data.

APPEARS THIS WAY  
ON ORIGINAL

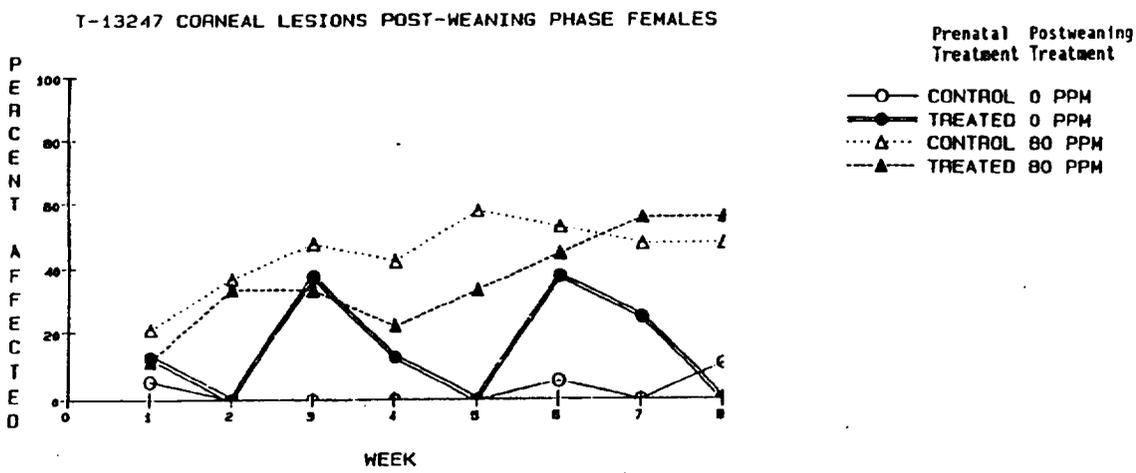
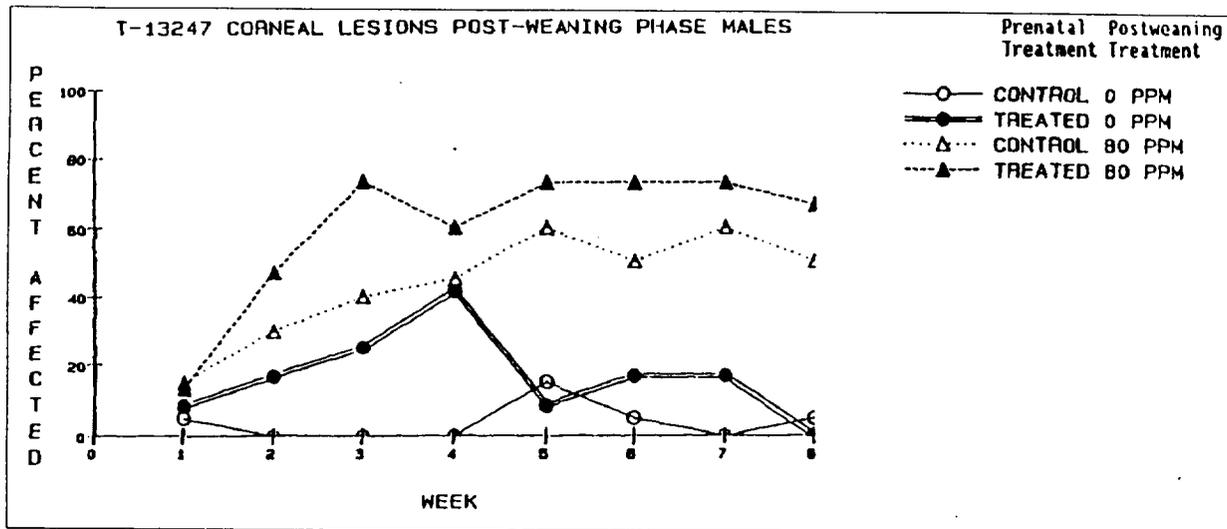
Sponsor's Table Vol. 1.6/126

T-13247: SC-0735 CROSS-FOSTERING REPRODUCTION PROBE IN RATS		
SUMMARY OF FEMALE OPHTHALMIC EXAMINATIONS		
FEMALES	DOSE LEVEL (MG/KG/DAY)	
	<u>0</u>	<u>100</u>
<u>NON-GRAVID</u>		
EXAMINED	7	5
NORMAL	6	3
CORNEAL OPACITIES		
LINEAR	1	0
FOCAL	0	2
<u>EARLY TERMINATIONS (- PPO 4)</u>		
EXAMINED	6	11
NORMAL	6	4
CORNEAL OPACITIES		
FOCAL	0	5
EXTENSIVE	0	2
<u>PRIOR TO TERMINAL SACRIFICE-GRAVID</u>		
EXAMINED	21	19
NORMAL	19	1
CORNEAL OPACITIES		
LINEAR	1	0
EXTENSIVE	0	6
EXTENSIVE WITH VASCULARIZATION	0	12
IRIS CONGESTED	1	0

No statistical analyses were conducted on these data.

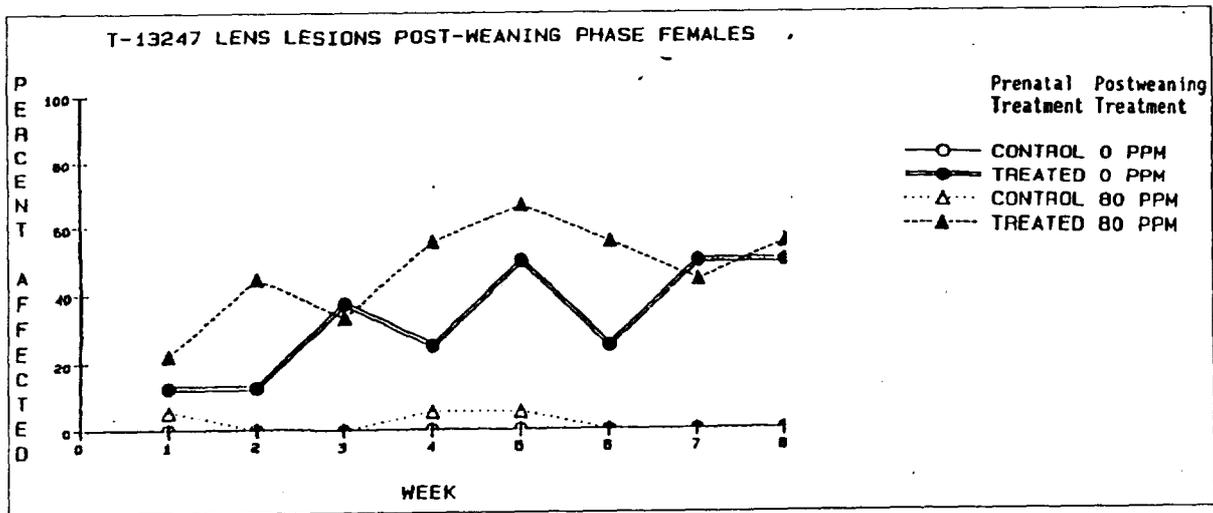
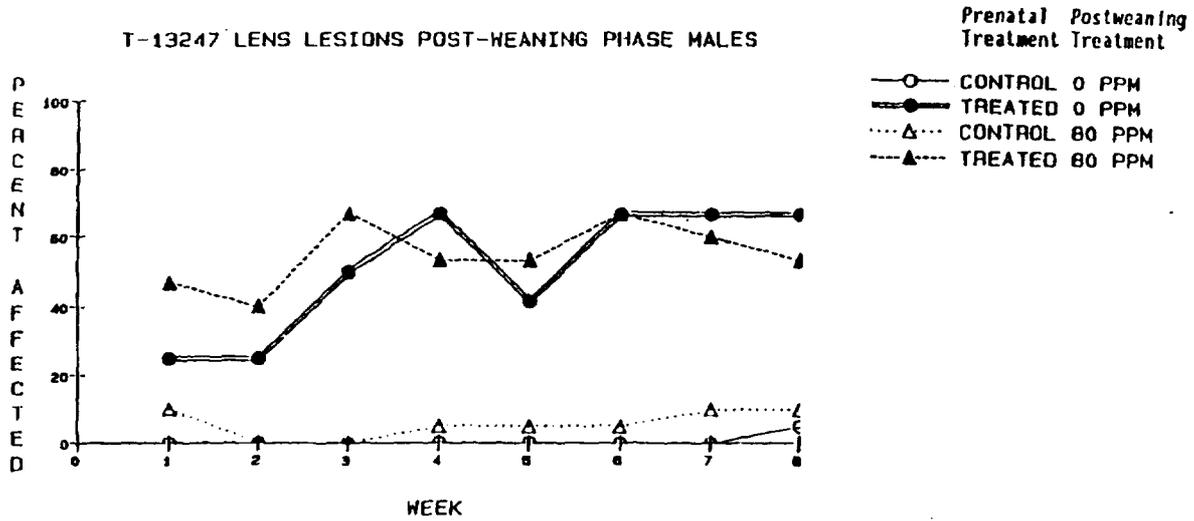
APPEARS THIS WAY  
ON ORIGINAL

Sponsor's Tables: Males - Vol. 1.6/128; Females - Vol.1.6/129



# BEST POSSIBLE COPY

Sponsor's Tables: Males - Vol. 1.6/130; Females - Vol. 1.6/131.



APPEARS THIS WAY  
ON ORIGINAL

**Summary and Evaluation:**

A cross-fostering reproduction probe was carried out in rats with SC-0735 at doses of 0 and 100 mg/kg.

Dams treated with 100 mg/kg SC-0735 showed maternal toxicity consisting of an increased incidence of corneal opacities and increased relative liver and kidney weights. Body weight, body weight gain and food consumption were reduced. Pups that received exposure by the intrauterine route showed a reduced survival, reduced mean pup weights and delayed eye opening. When pups were exposed to SC-0735 through lactation, mean pup weights were reduced and weanlings developed corneal opacities.

In addition lens lesions developed in animals with prior intrauterine compound exposure, regardless of whether or not they were exposed to drug in the diet during the 8 wk postweaning part of the study.

Corneal lesions were produced in animals during the postweaning exposure to SC-0735 regardless of whether or not they had been exposed to the compound prenatally.

**GENETIC TOXICOLOGY**

**Study Title:** NTBC Purified: Bacterial Mutagen Assay in *S. Typhimurium* and *E. Coli*

**Study No:** Report —/P/6300; — . Study No.: YV4404; Reference Nos.: Sponsor C08220;

— Test Subs.Y10506/001

**Study Type:** Ames - Bacterial Reverse Mutation Assay.

**Volume # and Page #:** Vol. 1.6 p 165

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Initiation/completion:** 06 May 99; experimental phase started 10 May 99; completed 27 May 99. Report dtd. 27Jul 99.

**GLP Compliance:** OECD Principles of Good Laboratory Practice (Ref. 471), revised 1997. (ENV/MC/CHEM(98)17). Except: The stability, homogeneity and achieved concentration of the test substance and control substances in the vehicles used were not determined by analysis (reported as due to short-term nature of studies) and certified purity and stability of the control substance was not available (by —). ICH S2A and S2B

[Reported that the test substance was within the stated expiry date; and the purity and characterization of the test substance were the responsibility of the Sponsor. Concentration refers to the concentration of test substance not corrected for purity.]

**QA- Reports Yes (X) No ( ):**

**Drug Batch Number:** 10912/94 Purity —

**Methodology:**

- **Strains/Species/Cell line:** *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA 100)
- *Escherichia coli* (WP2P and WP2P uvrA) in the presence and absence of a rat liver – derived metabolic activation system (S9-mix). [Well established assay designed to detect reversion to amino acid independence ( $his^-$  to  $his^+$  for *S. typhimurium*;  $trp^-$  to  $trp^+$  for *E. coli*) induced by chemicals that cause base changes or frameshift mutations in the genome of these organisms.
- **Dose Selection Criteria:** 100 - 5000  $\mu$ g/plate in the presence and absence of S9-mix.
- Metabolic Activation System:** S9-mix prepared from phenobarbital/ $\beta$ naphthoflavone induced Sprague-Dawley (CD) rats
- **Controls:**
  - **Vehicle:** DMSO
  - **Positive Controls:**

Acridine Mutagen ICR 191; Benzo[a]pyrene (BP);	[Solvent DMSO]
2-Aminoanthracene (2AA); Daunomycin HCL (DR);	[Solvent DMSO]
N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG);	[Solvent DMSO]
Mitomycin C (MMC); Sodium Azide (NaZ)	[Solvent H <sub>2</sub> O]