

| | | | | | | | | | | |
|-----------------|----|----|----|----|----|----|----|----|----|----|
| Sternum | | X | X | X | | X | X | X | X | X |
| Stomach | X | X | X | X | X | X | X | X | X | X |
| Testes | X* |
| Thymus | X* |
| Thyroid | X* | X | X | X | X* | X* | X* | X* | X* | X |
| Tongue | X | X | X | X | X | X | X | X | X | |
| Trachea | X | X | X | X | X | X | X | X | X | X |
| Urinary bladder | X | X | X | X | X | X | X | X | X | X |
| Uterus | X* | | X* | X* | X* | | X* | X* | X* | X |
| Vagina | X | | X | X | X | | | | | |
| Zymbal gland | | | | | | | | | | |

X, histopathology performed
 *, organ weight obtained

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 ON ORIGINAL**

2.6.6.4 Genetic toxicology

Study title: Bacterial mutagenicity report

Key findings:

- This was a mini-screen bacterial mutagenicity study
- Inconclusive results seen with the TA100 strain of *Salmonella typhimurium* with S9 activation

Study no.: RD1999/01825/00

Volume #, and page #: Module 4.2.3.3.1.1
Conducting laboratory and location: Glaxo Wellcome, Inc.
Date of study initiation: 9 June 1999
GLP compliance: Not included
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016B, Lot # U12816/67/1, purity unknown

Methods

Strains/species/cell line:

Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537
Escherichia coli strain WP₂uvrA

Concentrations used in definitive study:

10, 20, 25, 50, 100, 200, 400, 800 µg/plate

Basis of concentration selection:

No basis given

Negative controls:

DMSO

Positive controls:

| Strain | Without S9 | With S9 Activation |
|----------------------|-----------------|------------------------------------|
| TA1535 | sodium azide | All strains – 2-aminoanthracene |
| TA1537 | 9-aminoacridine | |
| TA98 | hycanthone | |
| TA100 | sodium azide | |
| WP ₂ uvrA | ENNG | |

Incubation and sampling times:

Details of study not given

Results

Study validity:

As this was a preliminary screen, insufficient details were given about the study to determine the validity of the methods used

Study outcome:

Negative results were found in each strain when tested without S9 activation

Negative results were found in all but the TA100 strain when tested with S9 activation

Inconclusive results in the TA100 strain when tested with S9 activation

Study title: GW572016F: *Salmonella* and *E. coli* Microsome standard plate incorporation assay (study V40754)

Key findings:

- GW572016F was not mutagenic under the conditions of this study

Study no.: RD2000/00409/01

Volume #, and page #:

Module 4.2.3.3.1.2

Conducting laboratory and location:

Glaxo Wellcome, Inc.

Medicines Safety Evaluation Division

& Bioanalysis and Drug Metabolism Division

5 Moore Drive

Research Triangle Park, NC 27709

Date of study initiation:

29 May 2000

GLP compliance:

Compliance letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

GW572016F, Lot # U14572/39/3, not given

Methods

Strains/species/cell line:

Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537

Escherichia coli strain WP_{2uvrA}

Concentrations used in definitive study:

100, 250, 500, 1000, 2500 µg/plate

Basis of concentration selection:

A range finding study was conducted to determine the solubility of the test article in the overlay agar and cytotoxicity of the test article. Concentrations of 100, 500, 1000, 2500, and 5000 µg/plate were used with strains TA98, TA100, TA1535, TA1537 and WP_{2uvrA} with and without S9 activation. The 5000 µg/plate had insoluble compound in the range finding assay and the concentration was therefore not used in the confirmatory assay.

There was some evidence of toxicity and precipitate in concentrations of 500 µg/plate and higher.

Negative controls:

DMSO

Positive controls:

| Strain | Without S9 | With S9 Activation |
|----------------------|-----------------|--------------------|
| TA1535 | sodium azide | |
| TA1537 | 9-aminoacridine | All strains – |
| TA98 | hycanthone | 2-aminoanthracene |
| TA100 | sodium azide | |
| WP ₂ uvrA | ENNG | |

Incubation and sampling times:

Incubated for 62-68 hours and then revertant colonies counted

Results

Study validity:

- Three replicate plates used in the confirmatory study
- Methods state that counts were done by hand and by automated counter and that the vehicle and control plates were counted only by automated counter, but details about the make or model of the automated counter are not given.
- Criterion for a positive result is the same as the criterion used for a positive control: TA98, TA100 or WP₂uvrA have a concentration that produces a mean reversion frequency that is two times or more greater than the mean reversion frequency of the corresponding vehicle control plates and the response is concentration-dependant or if TA1535 or TA1537 has a concentration that produces a mean reversion frequency that is three times or more greater than the mean reversion frequency of the corresponding vehicle control plates and the response is concentration-dependant.
- The negative and positive control values were within the historical control data ranges.
- Study design is valid. Though no evidence suggests a specific requirement of either a two- or three-fold increase in revertant colonies over background for a positive result, this is the generally accepted method of evaluation. The results showed both the positive and negative controls to be well within the historical controls, and the plates treated had comparable results to the negative controls.

Study outcome:

GW572016F (the ditosylate monohydrate salt form of GW572016) was not mutagenic in the microbial reverse mutation assay with or without S9 activation at the concentrations up to 5000 µg/plate in the range-finding assay and concentrations up to 2500 µg/plate in the confirmatory assay.

Study title: Mouse lymphoma mutagenicity report (non-GLP)

Key findings:

- Equivocal results seen in the 3-hr assay without activation
- The 3-hr assay with activation could not be evaluated
- Negative for mutagenicity in the 24-hr assay without activation

Study no.: RD2000/00306/00

| | |
|--|---------------------------------|
| Volume #, and page #: | Module 4.2.3.3.1.3 |
| Conducting laboratory and location: | Glaxo Wellcome, Inc. |
| Date of study initiation: | 11 May 1999 |
| GLP compliance: | No |
| QA reports: | yes () no (X) |
| Drug, lot #, and % purity: | GW572016B, Lot # U12816/67/1, — |

Methods

Strains/species/cell line:

L5178Y/*tk*^{+/−}-3.7.2C mouse lymphoma cells

Concentrations used in definitive study:

3-hr assay without S9 activation – 10, 20, 30, 40, 50, 60, and 70 µg/mL

24-hr assay without S9 activation – 2, 3.5, 5, 6.5, 8, 9.5, 11, and 12.5 µg/mL

3-hr assay with S9 activation – 25, 50, 75, 100, 200, 300, 400, and 500 µg/mL

Basis of concentration selection:

Concentrations for the confirmatory 3-hr assay were based on the toxicity seen in the first assay. The 3-hr assay was repeated because only two concentration levels in the first assay could be cloned due to toxicity. The 24-hr assay was only run once. The assay with S9 activation could not be completed due to the precipitation of the test article and the fact that the desired toxicity could not be reached. The highest concentration tested in the mouse lymphoma assay should yield a relative total growth (RTG) of 10-20%. This toxicity level was seen with the assays without S9 activation.

Severe cytotoxicity was seen at 60 µg/mL and higher in the first 3-hr assay, at 40 µg/mL in the repeat 3-hr assay and at 12.5 µg/mL in the 24-hr assay without S9 activation and these concentrations were not clonable. Significant toxicity was also seen in the first 3-hr assay at 50 µg/mL and at 30 µg/mL in the repeat 3-hr assay. In the 24-hr assay without S9 activation, significant toxicity was seen at 11 µg/mL. Test article precipitation was seen in the system without S9 activation at a concentration ≥ 50 µg/mL and with S9 activation at a concentration ≥ 75 µg/mL.

Negative controls:

DMSO

Positive controls:

Without S9 activation – Methanesulfonic acid methyl ester (MMS)

With S9 activation – 3-methylcholanthrene (3-MCA)

Incubation and sampling times:

Incubation and sampling times not given

Results

Study validity:

- Study was non-GLP
- Assay with S9 activation was not completed due to problems with precipitate and the lack of desired toxicity
- Criterion for a positive response is an increase in mutant frequency of at least 100×10^{-6} above the background mutant frequency
- Positive and negative controls were within normal ranges

Study outcome:

The RTG at the highest concentration tested at 24-hrs was 12%. In the 24-hr assay, without activation, there was no increase in mutagen frequency above background. The RTG at the highest concentration tested at 3-hrs without activation was 12% in the first assay and 9% in the second. There was an increase in mutant frequency in the 3-hr assay of 94×10^{-6} at 30 $\mu\text{g/mL}$ in the first assay and of 97×10^{-6} at 10 $\mu\text{g/mL}$ in the second assay. This result is equivocal in the 3-hr assay, as no concentration response was seen in the first or second assay.

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Study title: GW572016F: *In vitro* assay for chromosomal aberrations in Chinese Hamster Ovary (CHO) cells (study V40736)

Key findings:

- GW572016F did not induce chromosomal aberrations
- Increases in polyploidy and endoreduplication were seen in two of the assays

Study no.: RD2000/00577/00

Volume #, and page #:

Module 4.2.3.3.1.4

Conducting laboratory and location:

& Bioanalysis and Drug Metabolism Division
5 Moore Drive
Research Triangle Park, NC 27709

Date of study initiation:

17 March 2000

GLP compliance:

Compliance letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

GW572016F, Lot # U14572/39/3, —

Methods

Strains/species/cell line:

Chinese hamster CHO-K₁-BH₄ cell line

Concentrations used in definitive study:

1.25, 2.50, 5.00, and 750 µg/mL without S9 activation

5.00, 10.0, 20.0, and 25.0 µg/mL with S9 activation

Basis of concentration selection:

An initial study was conducted to examine for precipitation. In this assay, the highest concentration tested is usually either 5000 µg/mL or the highest concentration where significant toxicity is noted. Significant toxicity is generally defined as a reduction in mitotic index (MI) and/or the cell count to a level of approximately ≤ 50% of control.

Negative controls:

DMSO

Positive controls:

Mitomycin C (MMC) – without metabolic activation

Cyclophosphamide (CP) – with activation

Incubation and sampling times:

Initial trials

Incubation was for 3 hrs with drug treatment and 20.2 hr harvest in one assay and 17.8 hrs with drug treatment and 20.2 hr harvest in another in the initial assay without activation

Incubation was for 3 hrs with drug treatment and 20.2 hr harvest

Confirmatory trials

Drug exposure times were the same as in the initial trials but the cells were harvested after 44 hrs

Results

Study validity:

- Two replicate plates/concentration, with at least three analyzable test article concentrations.
- Criterion for a positive result is at least one concentration produces a significant increase in the number of cells with chromosomal aberrations and a concentration-response was observed.
- The negative and positive control values were within the historical control data ranges.
- Study design and results were valid

Study outcome:

- No increase in chromosomal aberrations was seen in the initial or confirmatory trials, with or without S9 activation.
- A significant increase in polyploidy was seen at two concentration levels in the initial trial with 3-hr treatment time and without S9 activation
- A significant increase in endoreduplication was seen at five concentration levels in the initial trial with 3-hr treatment time and with S9 activation
- GW572016F was negative for inducing chromosomal aberrations under the test conditions used in this study

**APPEARS THIS WAY
ON ORIGINAL**

Study title: GW572016F: *In vitro* assay for chromosomal aberrations in cultured human peripheral blood lymphocytes (Study V40806)

Key findings:

- No increase in chromosomal aberrations, polyploidy or endoreduplication

Study no.: RD2000/01529/00

Volume #, and page #:

Module 4.2.3.3.1.5

Conducting laboratory and location:

& Bioanalysis and Drug Metabolism Division
5 Moore Drive
Research Triangle Park, NC 27709

Date of study initiation:

19 June 2000

GLP compliance:

Compliance letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

GW572016F, Lot # U14572/39/3.

Methods

Strains/species/cell line:

Human peripheral lymphocytes from healthy donors

Concentrations used in definitive study:

5.00, 7.50, 10.0, 15.0, 20.0, and 25.0 µg/mL without S9 activation

5.00, 7.50, 10.0, 15.0, 20.0, 25.0, 30.0, and 40.0 µg/mL with S9 activation

Basis of concentration selection:

Concentrations for the confirmatory assay were based on an initial assay, with 3- and 19-hr treatment periods without metabolic activation and a 3-hr treatment period with metabolic activation.

Negative controls:

DMSO

Positive controls:

Mitomycin C (MMC) – without metabolic activation

Cyclophosphamide (CP) – with activation

Incubation and sampling times:

Cells were incubated with the drug for 3 and 19 hrs and harvested after 22 hrs in the initial trial without metabolic activation

In the confirmatory trial without metabolic activation, cells were treated for 19 hrs and harvested at 46 hrs

With metabolic activation, cells were treated for 3 hrs and harvested after 22 hrs in the initial trial and after 46 hrs in the confirmatory assay

Results

Study validity:

- Two replicate plates/concentration, with at least three analyzable test article concentrations.
- Criterion for a positive result is at least one concentration produces a significant increase in the number of cells with chromosomal aberrations and a concentration-response was observed.
- The negative and positive control values were within the historical control data ranges.
- Study design and results were valid

Study outcome:

Under the conditions of this test, GW572016F did not induce chromosomal aberrations or increase polyploidy or endoreduplication, with or without metabolic activation, in human peripheral blood lymphocytes.

Study title: GW572016F: Chromosomal aberrations *in vivo* in rat bone marrow cells (Study R40807)

Key findings:

- No increase in chromosomal aberrations seen

Study no.: RD2000/01601/00

Volume #, and page #:

Module 4.2.3.3.2.1

Conducting laboratory and location:

& Bioanalysis and Drug Metabolism Division
5 Moore Drive
Research Triangle Park, NC 27709

Date of study initiation:

22 June 2000

GLP compliance:

Compliance letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

GW572016F, Lot # U14572/39/3, —

Methods

Strains/species/cell line:

Sprague Dawley Rats — CD@ (SD) IGS BR).

Doses used in definitive study:

30, 150, 600, and 2000 mg/kg for male rats

5, 25, 100, and 2000 mg/kg for female rats

Basis of dose selection:

Based on toxicology studies showing higher plasma levels in female rats than males, the females were administered different doses than the male rats.

The three lower doses were designed to achieve 1X, 5X and 20X the IC90 value of 800 ng/mL and the highest dose, 2000 mg/kg, is the highest dose generally used in this assay.

Negative controls:

0.5% hydroxypropyl methylcellulose and 0.1% Tween 80

Positive controls:

Cyclophosphamide (CP)

Incubation and sampling times:

Animals were euthanized and bone marrow harvested at 18 and 42 hrs after single dose administration of GW572016F.

Results

Study validity:

- 100 cells from each rat were read.
- Slides were scored for micronuclei by eye.
- Criterion for a positive result is a significant elevation in structural chromosome damage in the treated animals when compared to vehicle control.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

Under the conditions of this assay, GW572016F did not induce chromosomal aberrations in rat bone marrow

Study title: — Reverse mutation assay "Ames test" using *Salmonella typhimurium*

Key findings:

- Designed to examine the mutagenicity of an impurity that chemical analysis shows is present at a level of no greater than $\mu\text{g/g}$ in the final drug product
- In 4 of 5 strains tested, when S9 metabolic activation was present. — was positive for mutagenicity under the conditions tested in this assay

Study no.: RD2005/00328/00

Volume #, and page #:

Module 4.2.3.7.6.3

Conducting laboratory and location:

Date of study initiation:

10 July 2002

GLP compliance:

Letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

— Lot 0203021-E, purity not given

Methods

Strains/species/cell line:

Salmonella typhimurium strains TA98, TA100, TA102, TA1535 and TA1537

Concentrations used in definitive study:

5, 15, 50, 150, 500, and 1500 $\mu\text{g/plate}$

Basis of concentration selection:

Based on a preliminary toxicity study with concentrations up to 5000 $\mu\text{g/plate}$ used with the TA100 strain of *Salmonella typhimurium*. The test material was toxic at concentrations of 500 $\mu\text{g/plate}$ and higher and at 1500 $\mu\text{g/plate}$ and higher no bacterial background was seen.

Negative controls:

DMSO

Positive controls:

| Strain | Without S9 | With S9 Activation |
|--------|-----------------|--------------------|
| TA1535 | EENG | 2-aminoanthracene |
| TA1537 | 9-aminoacridine | 2-aminoanthracene |
| TA98 | 4NQO | Benzo(a)pyrene |
| TA100 | EENG | 2-aminoanthracene |
| TA102 | Mitomycin C | DAN |

Incubation and sampling times:

Incubated for 48 hrs

Results

Study validity:

- Three replicate plates used in the confirmatory study
- Revertant colonies were counted using a colony counter and examined for effects on the growth of the bacterial background lawn
- Criterion for a positive result: a reproducible, concentration-related statistically significant increase in the revertant count in at least one strain of bacteria
- The negative and positive control values were within the historical control data ranges
- Study design is valid.

Study outcome:

Test material caused a visible reduction in background bacteria growth in all strains with and without S9 activation at concentrations of 500 µg/plate and above

A concentration related reproducible statistically significant increase in revertant colony frequency was recorded in the TA98, TA100, TA102 and TA1535 strains with metabolic activation at concentrations of 5 µg/plate and higher

No change in revertant colony frequency was seen in any strains when tested without metabolic activation

Study title: Screening L5178Y TK+/- mutation assay

Key findings:

- Designed to examine the mutagenicity of an impurity that chemical analysis shows is present at a level of no greater than — µg/g in the final drug The test article was considered to be mutagenic under the conditions of the assay

Study no.: RD2005/00329/00

Volume #, and page #:

Module 4.2.3.7.6.4

Conducting laboratory and location:

Date of study initiation:

23 February 2001

GLP compliance:

Letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

— Lot # S00L490, purity not given

Methods

Strains/species/cell line:

L5178Y/tk^{+/+}-3.7.2C mouse lymphoma cells

Concentrations used in definitive study:

3-hr assay without S9 activation – 3.75, 7.5, 15, 22.5, 30, and 45 µg/mL

24-hr assay without S9 activation – 2, 4, 8, 16, 24, and 32 µg/mL

3-hr assay with S9 activation – 3.75, 7.5, 15, 30, 45, and 60 µg/mL

Basis of concentration selection:

Concentrations for the confirmatory assays were based on the toxicity seen in preliminary assays. A steep concentration-related reduction in the relative suspension growth was seen with and without S9 activation. No precipitate of test article was noted at the preliminary concentrations, up to 40 µg/mL without S9 activation and 60 µg/mL with S9 activation. The highest concentration tested in the mouse lymphoma assay should yield a relative total growth (RTG) of 10-20%. This toxicity level was seen with the test article only after 24-hr exposure with and without S9 activation in the definitive study. Though with the 3-hr assays evidence of test material toxicity was seen by the % relative suspension growth (%RSG) and the Day 2 viability (%V).

Negative controls:

DMSO

Positive controls:

Without S9 activation – Ethylmethanesulphonate (EMS)

With S9 activation – Cyclophosphamide (CP)

Incubation and sampling times:

Incubated for 3 hrs and treated with the drug for 3 or 24 hrs

ResultsStudy validity:

- Criterion for a positive control is a marked increase in mutant frequency above control of at least approximately 5-fold increase but preferably 10-fold or greater
- A positive response for the test article is considered if two of the following are met – (1) a greater than 3-fold increase in mutant frequency per survivor over the vehicle control value, (2) a concentration-related increase in the mutant frequency per survivor and (3) an increase in the absolute number of mutants. If only one of these is met, the response is equivocal.
- Positive and negative controls were within normal ranges
- Study is valid

Study outcome:

At the 3-hr exposure period, with or without S9 activation, the RTG did not indicate optimum toxicity of the test article. Adequate levels of toxicity were seen in the 24-hr exposure rate studies. At 24-hrs, with S9 activation, the test article induced a significant concentration-related increase in mutant frequency.

Study title: — Micronucleus test in the mouse

Key findings:

- Designed to test the potential for an impurity that chemical analysis shows is present at a level of no greater than — $\mu\text{g/g}$ in the final drug to produce chromosomal damage or aneuploidy
- The test article was considered to be genotoxic under the conditions of this test

Study no.: RD2003/01997/00

Volume #, and page #:

Module 4.2.3.7.6.5

Conducting laboratory and location:

Date of study initiation:

16 January 2003

GLP compliance:

Compliance included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

— , lot # 0203021-E, purity not given

Methods:

Strains/species/cell line:

— CD-1TM(ICR)BR mouse

Doses used in definitive study:

100, 200 and 400 mg/kg

Basis of dose selection:

Doses were chosen based on a rang-finding toxicity study where doses of 400, 600 and 800 mg/kg were given to mice in a single oral administration. This study showed 800 mg/kg to be too toxic, as mice were euthanized moribund. It was determined that sufficient toxicity was seen with the 400 mg/kg dose (labored respiration, ↓ respirations, lethargy, ataxia, ptosis, dehydration, hypothermia, hunched posture, splayed gait and pilo-erection) and 400 mg/kg was determined to be the MTD. It was also determined that no gender differences were noted so the main study was conducted with only male mice.

Negative controls:

Arachis oil

Positive controls:

Cyclophosphamide (CP)

Incubation and sampling times:

Mice were euthanized 24 and 48 hrs after drug administration and slides were prepared following termination.

Results:

Study validity:

- Two slides were prepared from each mouse
- Slides were scored for micronuclei by eye.
- Criterion for a positive result is a significant, dose-related and toxicologically relevant increase in the number of micronucleated polychromatic erythrocytes (MNPCE) in the treated animals when compared to control.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

At 400 mg/kg both slides analyzed showed a statistically significant increase in MNPCE over control. The 200 mg/kg slides showed a marked, though not statistically significant increase in MNPCE over control. When results of both slides for each mouse were combined, the effect at 200 mg/kg was also statistically significant, leading to the conclusion that under the conditions of this test, the metabolite of GW572016, — was genotoxic.

Study title: — Oral rat bone marrow micronucleus assay

Key findings:

- Designed to examine the potential of an impurity that chemical analysis shows is present at a level of no greater than — µg/g in the final drug to produce structural chromosomal damage or aneuploidy *in vivo* in polychromatic erythrocytes from rat bone marrow
- The test article induced micronuclei in rats in an *in vivo* bone marrow nucleus assay, under the conditions of this test

Study no.: WD2005/00458/00

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| Volume #, and page #: | Module 4.2.3.7.6.6 |
| Conducting laboratory and location: | GlaxoSmithKline Hertfordshire, UK |
| Date of study initiation: | May 2005 |
| GLP compliance: | Compliance included and signed |
| QA reports: | yes (X) no (·) |
| Drug, lot #, and % purity: | — lot # 0000013743, purity not given |

Methods:

Strains/species/cell line:

SD(SD) rat

Doses used in definitive study:

10, 20, 100, 250, 500, 1000 and 2000 mg/kg dosed on Days 1 and 2

Basis of dose selection:

Doses were chosen based on several toxicology studies conducted with the GW572016 impurity. An acute study showed the LD50 to be between 500-2000 mg/kg/day. A 28-day repeated dose study showed that 1000 mg/kg/day was too toxic and animals were euthanized moribund on Day 8. Doses of 150 and 500 mg/kg/day showed methemoglobinemia and hemolysis.

Negative controls:

Polyethylene glycol

Positive controls:

Cyclophosphamide (CP)

Incubation and sampling times:

Bone marrow aspirated from rats after euthanized on Day 3, 24-hrs after last drug administration

Results:

Study validity:

- Slides were scored for micronuclei by eye.
- Criterion for a positive result is if any treatment group shows a mean frequency of MNPCE which is > 4 times the concurrent vehicle.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

There was no indication of erythrocyte toxicity, as %PCE values were comparable for all groups, including vehicle control. The first study used doses of 500, 1000 and 2000 mg/kg/day and this study indicated that — induced micronuclei (2-3 fold increase over control) with 4/6 rats at the LD showing >6MNPCE/2000 PCE, which is seen in less than 1% of animals in the control range. So although there was not a >4 fold increase in MNPCE/ 2000 PCE, the results were deemed positive.

Study title: — . Unscheduled DNA synthesis assay in rat hepatocytes following oral dosing

Key findings:

- Designed to examine the potential for an impurity that chemical analysis shows is present at a level of no greater than — $\mu\text{g/g}$ in the final drug to produce structural chromosomal damage or aneuploidy in vivo in polychromatic erythrocytes from rat bone marrow
- The test article was negative for unscheduled DNA synthesis (UDS) under the conditions of this test

Study no.: WD2005/00898/00

Volume #, and page #:

Module 4.2.3.7.6.7

Conducting laboratory and location:

Date of study initiation:

25 May 2005

GLP compliance:

Compliance included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

— lot # 0000013743, purity —

Methods:

Strains/species/cell line:

CD(SD) rat

Doses used in definitive study:

500, 1000 and 2000 mg/kg dosed on Days 1 and 2

Basis of dose selection:

Doses were chosen based on the rat micronucleus assay which used 2000 mg/kg/day as the highest acceptable dose. Only male rats were used, as previous toxicology studies showed no substantial gender differences in the rat. This is not exactly accurate, as the previous toxicology studies have shown higher plasma levels in female rats and in the long-term toxicology studies, doses used in female rats were lower than those used in male rats.

Negative controls:

Polyethylene glycol

Positive controls:

2-acetamidofluorene (2-AAF)

Incubation and sampling times:

Hepatocytes were obtained from rats after they were euthanized approximately 2-4 hrs after last of the two doses of drug administration (Day 2)

Results:

Study validity:

- Mean cell viability for scored cultures was not less than 50%
- Vehicle and positive control animals had a group mean (Net Nuclear Grain) count-*NNG value within or close to laboratory controls
- Positive control treatments had a group mean NNG value of at least 5, with 50% or more cells responding (NNG counts of 5 or more)
- Test article is considered to give a positive response if the group mean NNG value is 0 or greater with 20% or more cells in repair

Study outcome:

The results showed group mean NNG counts for the male rats to be 0.1 (500 mg/kg), 0.1 (1000 mg/kg) and 0 (2000 mg/kg) with no cells observed to be in repair at any dose level. This is similar to the results for the vehicle control (-0.1 NNG). Under conditions of this study, — was negative for UDS.

2.6.6.5 Carcinogenicity

Carcinogenicity studies in both the rat and mouse are ongoing with lapatinib

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2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: GW572016F: Oral male fertility study in rats

Key study findings:

- Decreased body weights in HD males bred to untreated females
- No effects on breeding parameters or caesarian sectioning data from the untreated females bred to treated males
- No gross fetal malformations noted, no effects on fetal body weights

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| Study no.: | CD/2002/00007/00 |
| Volume #, and page #: | Module 4.2.3.5.1.1 |
| Conducting laboratory and location: | Department of Safety Assessment GlaxoSmithKline King of Prussia, PA |
| Date of study initiation: | 17 August 2001 |
| GLP compliance: | Letter included and signed |
| QA reports: | yes (X) no () |
| Drug, lot #, and % purity: | GW572016F, Lot# R5361/44/1, purity not given though other studies have shown this Lot # with a purity around <u> </u> |

Methods

| | |
|---|--|
| Doses: | 20, 60 or 180 mg/kg/day |
| Species/strain: | Rat/Wistar –Han |
| Number/sex/group: | 25 males/dose |
| Route, formulation, volume, infusion rate: | PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 10 mL/kg volume |
| Satellite groups used for toxicokinetics: | None |
| Study design: | F0 males dosed from Day 1 until termination (Day 63-67), mated with untreated females after 28 doses. Females euthanized on Day 20 after mating |
| Parameters and endpoints evaluated: | Males: in-life observations, body weight, food consumption, mating, fertility, necropsy, organ weights Females: in-life observations, body weight, gross examination of uterus and cervix, uterus weight, corpora lutea, implantations, resorptions, placental morphology, live and dead fetuses F1 litters: fetal weight, sex and external morphology |

Results

Mortality:

One LD male was euthanized moribund on Day 53. Clinical signs included weight loss, crusty material around the eye, dehydration, lack of righting reflex, and lethargy. Necropsy revealed a cerebellar mass, approximately 3 mm and discolored. It is not believed to be drug-related, given the isolated incident at the lowest dose.

No other deaths were seen in the study

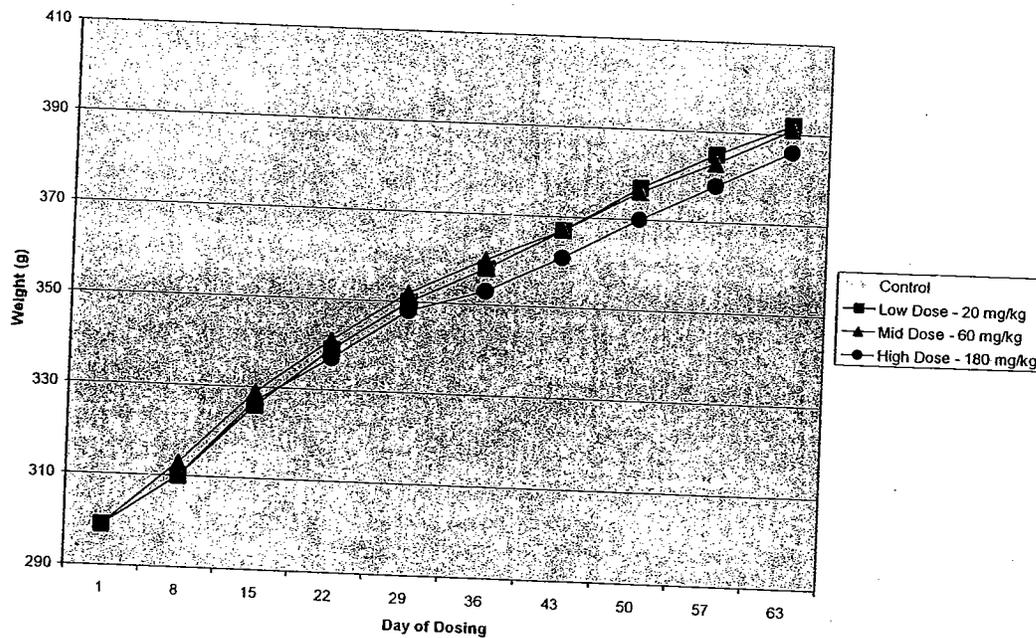
Clinical signs:

Increased salivation was seen, in a dose-related fashion, in male rats treated with GW572016.

Body weight:

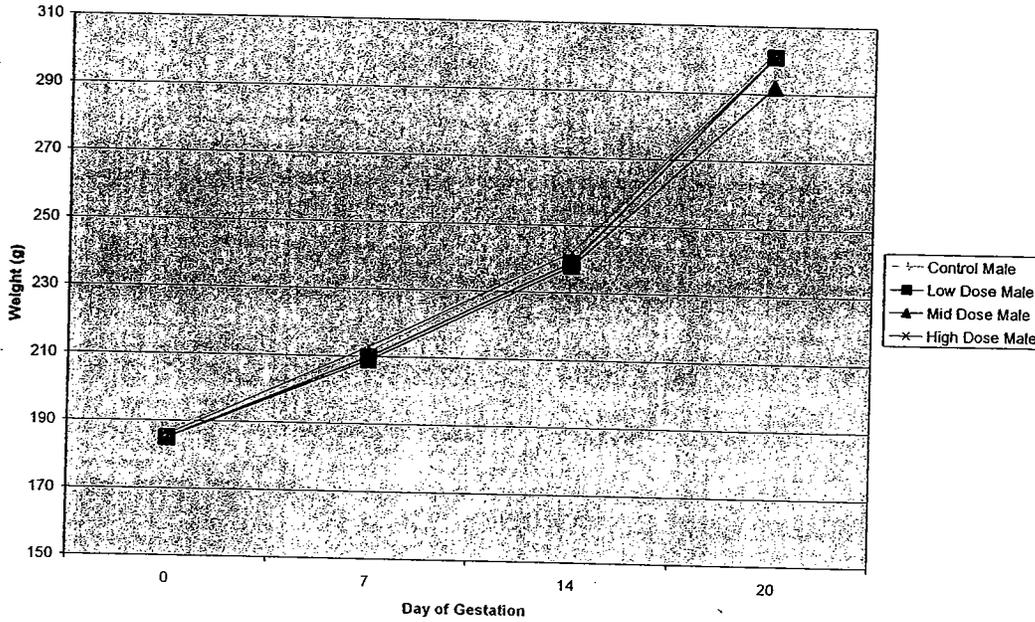
All the male F0 rats showed increased body weight throughout the study, with a significant difference between the body weights of the HD rats and that of the control rats.

Body Weights - Male Fertility Rats



The graph below shows the body weights of the pregnant rats that were not treated with GW572016 but bred to male rats that were. The graph shows that the pregnant females gained weight in comparable fashion irrespective of treatment group of the male breeder rats.

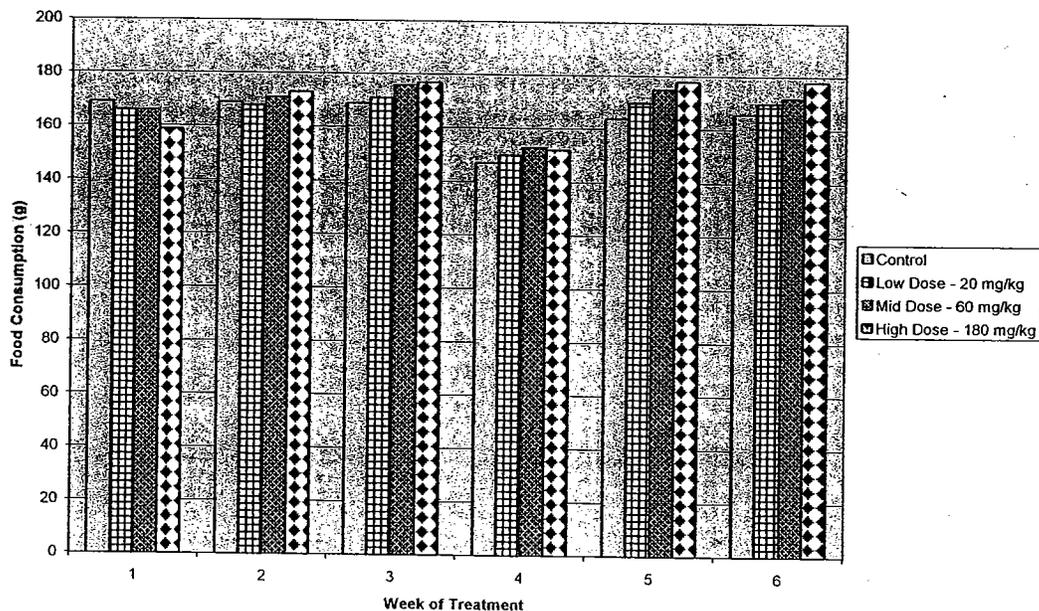
Weights of Pregnant Non-Treated Rats Bred to Treated Males



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ON ORIGINAL**

Food consumption:

Food consumption during the study are presented below, with significant differences between control and MD and HD rats during Week 5 and between control and HD rats during Week 6. During these time points, the control rats ate less food than the GW572016 rats. There is little toxicological relevance to these significant differences and previous studies have not shown GW572016 to increase food consumption.

Food Consumption - Male Fertility RatsToxicokinetics:

Not conducted

Necropsy:*F0 necropsy results*

No effect on the male organ weights was noted, including in the reproductive organs. The rats were examined for gross malformations and no drug-treatment related changes were seen.

F1 necropsy results

Only one incidence of external malformation was seen, and it was in a control group litter. Fetal body weights are shown in the table below, and no effect of the paternal drug treatment was noted.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 180 mg/kg |
|----------------------------|---------|----------------------|----------------------|------------------------|
| Male fetal weight - mean | 3.53 | 3.40 | 3.45 | 3.50 |
| Female fetal weight - mean | 3.68 | 3.60 | 3.64 | 3.72 |

Fertility parameters:

Mating parameters, presented in the table below, show that the drug exposure did not significantly impact the ability of the male rats to breed, to impregnate the untreated females, or the amount of time it took until mating.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 180 mg/kg |
|---------------------|---------|----------------------|----------------------|------------------------|
| Mating Incidence | 100 | 100 | 100 | 100 |
| Pregnancy Incidence | 96 | 92 | 100 | 88 |
| Days to Mating | 2.9 | 2.9 | 3.1 | 2.8 |

The pregnancy parameters are presented, as averages for each male dose group, in the table below. The only significant difference seen was in gravid uterine weights in the females bred to the MD males. While the females bred to MD males had a higher percentage of resorbed implantations and slightly less number of live fetuses, these parameters are not significant. The lowered gravid uterine weights in the MD-bred females is not likely toxicologically relevant given the lack of significant effects in the other parameters and the lack of a dose-response effect.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 180 mg/kg |
|-------------------------------|---------|----------------------|----------------------|------------------------|
| Number corpora lutea | 12.5 | 12.8 | 12.2 | 11.8 |
| Number implantations | 11.3 | 11.5 | 10.2 | 11.0 |
| Percent pre-implantation loss | 10.1 | 9.8 | 15.9 | 7.2 |
| Number of resorptions | | | | |
| Early | 0.5 | 0.7 | 0.8 | 0.3 |
| Late | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 0.5 | 0.7 | 0.8 | 0.3 |
| Percent implants resorbed | 4.6 | 5.5 | 7.4 | 2.8 |
| Number live fetuses – mean/♀ | 10.7 | 10.8 | 9.3 | 10.6 |
| Number live male fetuses | 5.3 | 5.1 | 5.1 | 5.8 |
| Number live female fetuses | 5.5 | 5.7 | 4.2 | 4.8 |
| Number of dead fetuses | 0 | 0 | 0 | 0 |
| Live birth index (%) | 100 | 100 | 100 | 100 |
| Gravid uterus weight (g) | 60.1 | 59.5 | 51.3* | 59.6 |

* - Statistically significant compared to control

Study title: GW572016F: Oral study of female fertility and early embryonic development to implantation in rats

Key study findings:

- Decreased body weights seen in HD females, and decreased body weight gain at the end of gestation seen in MD and HD
- No effects seen on mating or fertility index
- Decreased fetal body weights seen in MD and HD litters
- Increased resorptions, decreased live fetuses and gravid uterus weights seen in HD animals, with decreased gravid uterus weights also seen in MD rats
- External malformations noted in HD litters only, but relationship to drug-treatment is tenuous

Study no.: CD/2002/00032/00
Volume #, and page #: Module 4.2.3.5.1.2
Conducting laboratory and location: Department of Safety Assessment
 GlaxoSmithKline
 King of Prussia, PA
Date of study initiation: 17 August 2001
GLP compliance: Letter included and signed
QA reports: yes (X) no ()
Drug, lot #, and % purity: GW572016F, Lot# R5361/44/1, purity not given though other studies have shown this Lot # with a purity around —

Methods

Doses: 20, 60 or 120 mg/kg/day
 Lower HD than in the male fertility study due to ↑ toxicity seen in female rats

Species/strain: Rat/Wistar –Han

Number/sex/group: 25 females/dose

Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 10 mL/kg volume

Satellite groups used for toxicokinetics: None

Study design: F0 females dosed for 15 days before housing with breeder males, for up to 14 days during breeding, and GD 0-6. Females then euthanized on GD 20. F0 males were not dosed at all

Parameters and endpoints evaluated: Females: in-life observations, body weight, food consumption, estrous cycle, mating, fertility, necropsy, uterus weight, corpora lutea, implantations, resorptions, gross placental morphology, live and dead fetuses
 F1 litters: fetal weight, sex and external morphology

Results

Mortality:

No mortality due to drug-treatment was seen during this study

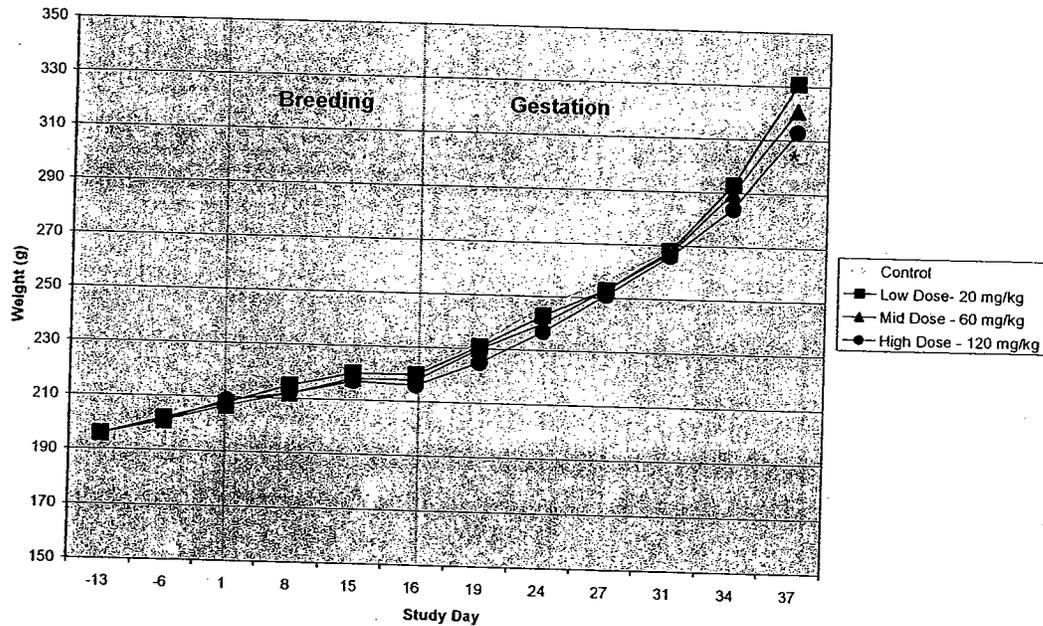
Clinical signs:

Clinical signs seen with more frequency in the drug-treated females were salivation and red staining and scabbing of the coat.

Body weight:

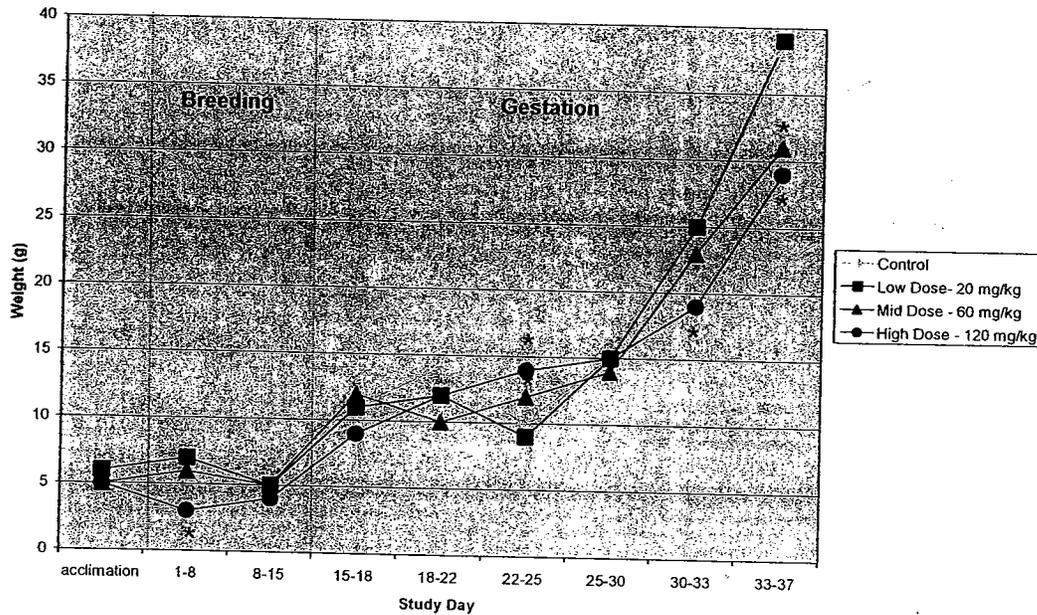
The body weights of the treated F0 females is shown in the chart below, with weights taken during acclimation, breeding and then during gestation. The only significant point, shown with an asterisk, is the weight of the HD females on the last day of the study, GD 20. Body weights of the HD females at this time point were significantly lower than the control rats.

Body Weights - F0 Treated Females



The graph below shows the body weight changes throughout the study in the pregnant rats. Of note is the fact that the weight gains in the MD and HD are significantly lower than control during the last stages of gestation, with the HD for the last 6 days, from GD 14 until GD 20, and for the MD from GD 17-20. In both the MD and HD rats, body weight gains were increased over control during the period after dosing ceased, GD 7. That is shown on the graph below as Study Day 22.

Body Weight Changes - F0 Treated Females

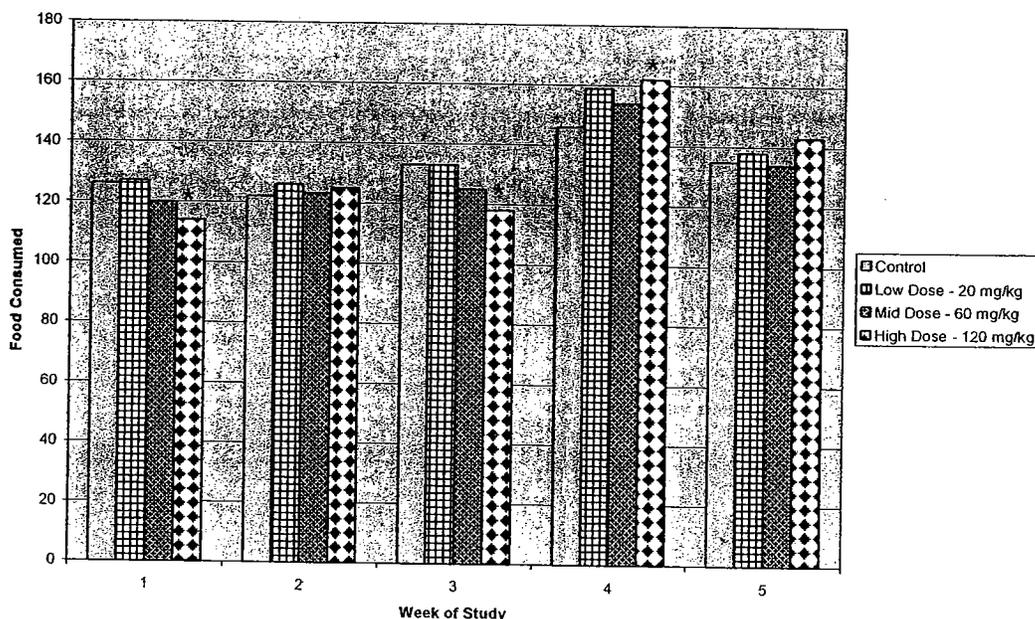


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Food consumption:

The food consumption of the treated females, during two weeks of breeding and the three weeks of gestation, is presented in the graph below. Significantly lower food consumption was seen in the HD rats during the Week 1 of the study and the Week 3, the first week of gestation, with significantly increased food consumption seen in the HD rats during Week 4, the second week of gestation. Significant data points are shown with an asterisk.

Food Consumption - F0 Treated Females

Toxicokinetics:

Not conducted

Necropsy:*F0 necropsy results*

The F0 female rats were examined for gross malformations and no drug-treatment related changes were seen.

F1 necropsy results

There were four incidences of fetal malformation seen and all were in the HD group. The pups with these malformations were in four different groups, leading to a significant number of litters with at least one malformation (4/22 HD litters and 0/24 controls).

- Exencephaly and protruding tongue
- Craniorachischisis, low set pinna and open eye
- Acephaly and omphalocele
- Omphalocele

It should be noted that these malformations all occurred in body areas/systems that develop after the time point that drug administration had ceased. Given that, it is unlikely

that it was drug-related, but given that no other drug group exhibited any malformations other than the HD GW572016 group, a drug effect can not be completely discounted. In pregnant rats, the $t_{1/2}$ of GW572016 was 7-9 hrs at the highest dose and a tissue distribution study in rats give [14 C]-GW572016 showed that the radioactivity was nearly cleared from the body within 24 hrs. Given that, it is unlikely that the circulating levels of drug were in the dams during the organogenesis.

Fetal body weights are shown in the table below, and the effect of drug treatment is evident in the reduced average fetal body weights in the MD and HD litters.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|----------------------------|---------|----------------------|----------------------|------------------------|
| Male fetal weight - mean | 3.61 | 3.59 | 3.31* | 3.18* |
| Female fetal weight - mean | 3.82 | 3.79 | 3.54* | 3.38* |

* - Statistically significant compared to control

Fertility parameters:

Mating parameters, presented in the table below, show that the drug exposure did not significantly impact the ability of the female rats to breed, to become pregnant, or the amount of time it took until mating. The average number estrous cycles were also equivalent across groups.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|--------------------------------------|---------|----------------------|----------------------|------------------------|
| Mating Index | 100 | 100 | 100 | 100 |
| Fertility Index | 96 | 96 | 100 | 92 |
| Days to Mating | 4.0 | 3.3 | 3.3 | 3.8 |
| Mean # Estrous Cycles/ 15 Days Tx | 2.8 | 2.9 | 2.8 | 2.7 |

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ON ORIGINAL

The pregnancy parameters are presented, as averages for each dose group, in the table below. As the data show, treatment of female rats during breeding and through the first 7 days of gestation led to significant effects, primarily in the HD group, on resorptions, live fetuses and gravid uterus weight. Treatment during this time period with HD GW572016 led to higher numbers of early resorptions and percent of implantations that were resorbed and to a decrease in the number of live fetuses, with only the number of male fetuses being significantly decreased. The lower number of live fetuses, and increases in absorptions, led to the significant decrease in gravid uterus weight. Gravid uterine weights were also significantly lower in the MD group. Though no other parameters were significant in this group, a trend toward more resorptions and decreased live fetuses was seen.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|-------------------------------|---------|----------------------|----------------------|------------------------|
| Number corpora lutea | 13.2 | 13.0 | 13.2 | 12.6 |
| Number implantations | 12.3 | 12.1 | 12.0 | 10.9 |
| Percent pre-implantation loss | 6.8 | 7.2 | 8.5 | 13.7 |
| Number of resorptions | | | | |
| Early | 0.8 | 0.5 | 1.4 | 3.1* |
| Late | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 0.8 | 0.5 | 1.4 | 3.1* |
| Percent implants resorbed | 6.1 | 4.5 | 11.2 | 30.9* |
| Number live fetuses – mean/♀ | 11.5 | 11.5 | 10.5 | 8.1* |
| Number live male fetuses | 5.5 | 5.9 | 4.8 | 3.5* |
| Number live female fetuses | 6.0 | 5.6 | 5.8 | 4.6 |
| Number of dead fetuses | 0 | 0 | 0 | 0 |
| Live birth index (%) | 100 | 100 | 99.7 | 100 |
| Gravid uterus weight (g) | 65.9 | 65.3 | 57.5* | 43.4* |

* - Statistically significant compared to control

**APPEARS THIS WAY
ON ORIGINAL**

Embryofetal development

Study title: GW572016F (ErbB2 inhibitor): Study to determine the maximum repeatable daily oral dose in pregnant Wistar Han rats.

Key study findings:

- HD of 180 mg/kg clearly too toxic for reproductive toxicity testing, all animals at this dose euthanized moribund, body weights and food consumption were decreased
- Plasma concentrations of GW572016 increase with increasing doses

Study no.: WD/2001/00235/00
Volume #, and page #: Module 4.2.3.5.2.1
Conducting laboratory and location: Glaxo Wellcome Research and Development
Ware, Hertfordshire, UK
Date of study initiation: 23 August 2000
GLP compliance: No
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016F, Lot# U14572/31/5, — purity

Methods

Doses: 5, 30, 60 or 180 mg/kg/day
Species/strain: Rat/Wistar –Han
Number/sex/group: 3 females/dose
Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose
+ 0.1% Tween 80, 10 mL/kg volume
Satellite groups used for toxicokinetics: None – blood taken from main rats on GD 7
and GD 16/17
Study design: F0 females dosed from GD 7-19, with the
day of mating noted as GD 1. Females then
euthanized on GD 20.
Parameters and endpoints evaluated: Females: in-life observations, body weight,
food consumption, gross necropsy, uterus
weight, corpora lutea, implantations,
resorptions, gross placental morphology,
live and dead fetuses, toxicokinetics

Results

Mortality (dams):

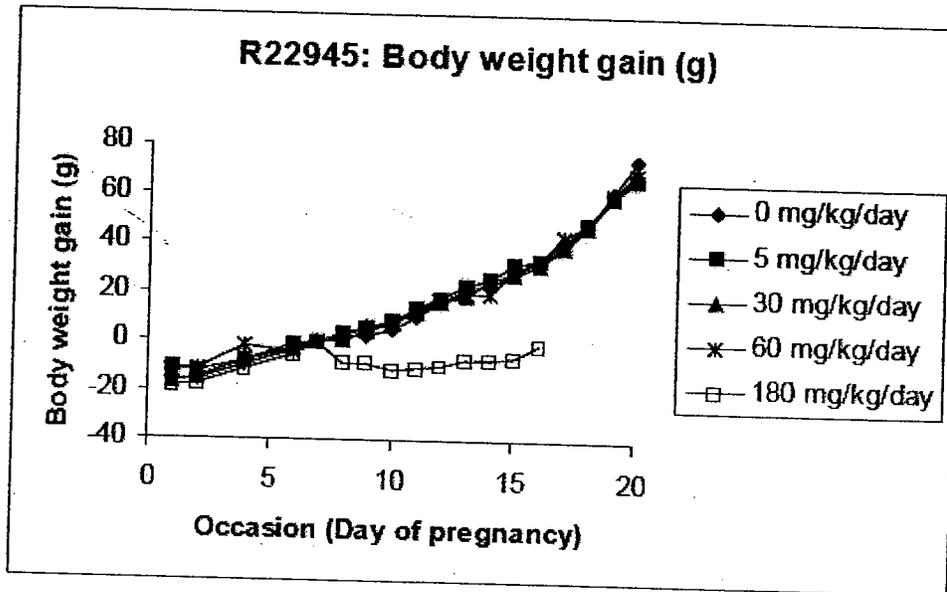
All the HD rats were euthanized moribund on GD 16 due to decreases in body weight gain and food consumption, as well as red vaginal discharge

Clinical signs (dams):

Clinical signs seen more frequently in the drug-treated groups included piloerection and persistent red vaginal discharge in the HD group.

Body weight (dams):

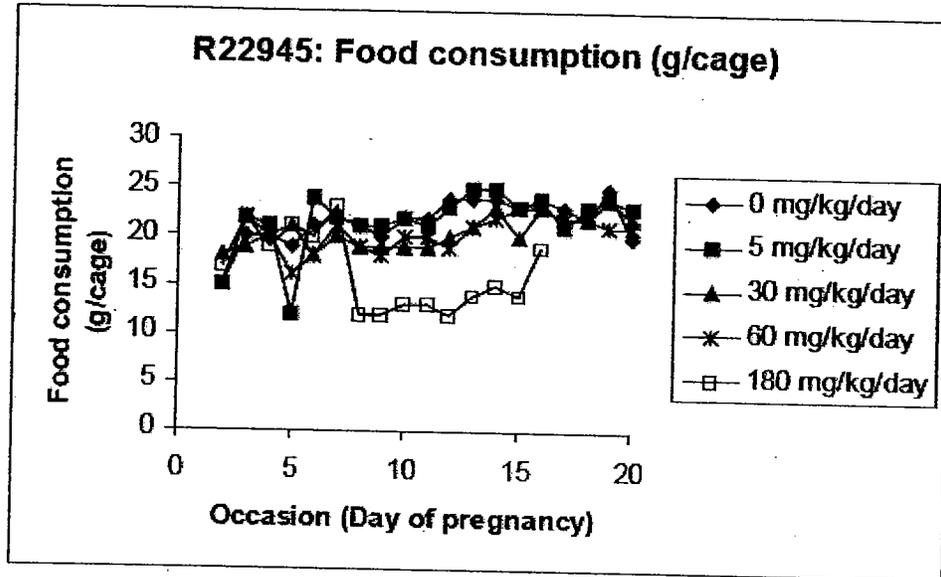
Body weights of the dams were adversely affected by GW572016 treatment, as is depicted in the sponsor's graph presented below. The HD rats, prior to being euthanized moribund, had clearly decreased body weights compared to all the other groups.



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Food consumption (dams):

The food consumption data, like the body weights, show that the HD rats were adversely affected by GW572016 treatment, with decreased food consumption seen. This is seen in the sponsor's graph, presented below.



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ON ORIGINAL

Toxicokinetics:

The sponsor's table below shows that detectable levels of GW572016 were present in all the drug-treated rats after the first dose of drug and after the eleventh dose of drug. These data show that the plasma concentrations also increase with the increasing dose of GW572016 the first day, and with most doses after the eleventh dose was administered.

Table iii Average Plasma Concentrations of GW572016X in Pregnant Female Rats at 8-hours after Single and Repeat Oral Administration of 0, 5, 30, 60, or 180mg GW572016X/kg/day.

| Day | Dose (mg/kg/day) | Average (ng/mL) | SD (ng/mL) |
|--------------------------|------------------|-----------------|------------|
| 7 (1 st Dose) | 0 | BQL | BQL |
| | 5 | 178 | 109 |
| | 30 | 3899 | 1556 |
| | 60 | 4922 | 2136 |
| | 180 | 31886 | 13716 |
| (11 th Dose) | 0 | BQL | BQL |
| | 5 | 282 | 144 |
| | 30 | 6154 | 3255 |
| | 60 | 5955 | 275 |
| | 180 | 31770 | 14557 |

BQL = Below the Quantitation Limit (10 ng/mL)

SD = Standard Deviation

* samples from animals at 180mg GW572016X/kg/day were collected 6.5 hours after dosing on Day 16 of pregnancy

Terminal and necroscopic evaluations:C-section data:

Results of the uterine examinations are presented in the table below. No information is available for the HD rats, as they were euthanized prior to the end of the study. The remaining doses did not adversely affect the parameters measured.

| | Control | Low Dose 5 mg/kg | Mid1 Dose 30 mg/kg | Mid2 Dose 60 mg/kg |
|--------------------------------|---------|---------------------|--------------------------|--------------------------|
| Number corpora lutea | 12.7 | 11.3 | 11.7 | 12.7 |
| Number implantations | 12.3 | 11.0 | 11.0 | 12.0 |
| Percent pre-implantation loss | 2.6 | 2.9 | 5.7 | 5.3 |
| Intrauterine deaths | | | | |
| Early | 0.3 | 0.0 | 0.3 | 0.0 |
| Late | 0.0 | 0.0 | 0.0 | 0.0 |
| Dead | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 0.3 | 0.0 | 0.3 | 0.0 |
| Percent post-implantation loss | 2.7 | 0 | 3.0 | 0 |
| Number live fetuses – mean/♀ | 12.0 | 11.0 | 10.7 | 12.0 |
| Live birth index (%) | 97.3 | 100 | 97.0 | 100 |

Offspring:

Not examined for malformations

Study title: GW572016F (ErbB2 inhibitor): A further study to determine the maximum repeatable daily oral dose in pregnant Wistar Han rats.

Key study findings:

- HD of 120 mg/kg was tolerated for dosing from GD 7-20 with minimal toxicity
- Decreases in body weight and slight decreases in food consumption seen in the HD rats
- No adverse effects seen on the caesarian sectioning data, therefore the HD in this study was chosen as the HD for the definitive embryo-fetal development study in the rat

| | |
|--|--|
| Study no.: | WD/2001/00236/00 |
| Volume #, and page #: | Module 4.2.3.5.2.2 |
| Conducting laboratory and location: | Glaxo Wellcome Research and Development Ware, Hertfordshire, UK |
| Date of study initiation: | 26 September 2000 |
| GLP compliance: | No |
| QA reports: | yes () no (X) |
| Drug, lot #, and % purity: | GW572016F, Lot# U14572/31/5, — purity |

Methods

| | |
|---|---|
| Doses: | 90 or 120 mg/kg/day |
| Species/strain: | Rat/Wistar –Han |
| Number/sex/group: | 3 females/dose |
| Route, formulation, volume, infusion rate: | PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 10 mL/kg volume |
| Satellite groups used for toxicokinetics: | None – blood taken from main rats on GD 7 and GD 17 |
| Study design: | F0 females dosed from GD 7-20, with the day of mating noted as GD 1. Females then euthanized on GD 21. |
| Parameters and endpoints evaluated: | Females: in-life observations, body weight, food consumption, gross necropsy, uterus weight, corpora lutea, implantations, resorptions, gross placental morphology, live and dead fetuses, toxicokinetics |

Results

Mortality (dams):

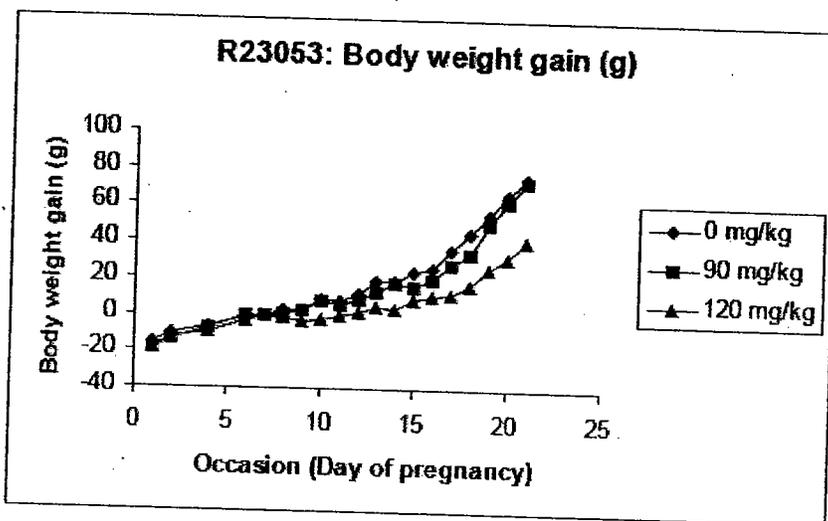
No maternal deaths during the study

Clinical signs (dams):

Clinical signs seen more frequently in the drug-treated groups included piloerection and one HD dam was lethargic, cold to the touch, pale and with hunched posture.

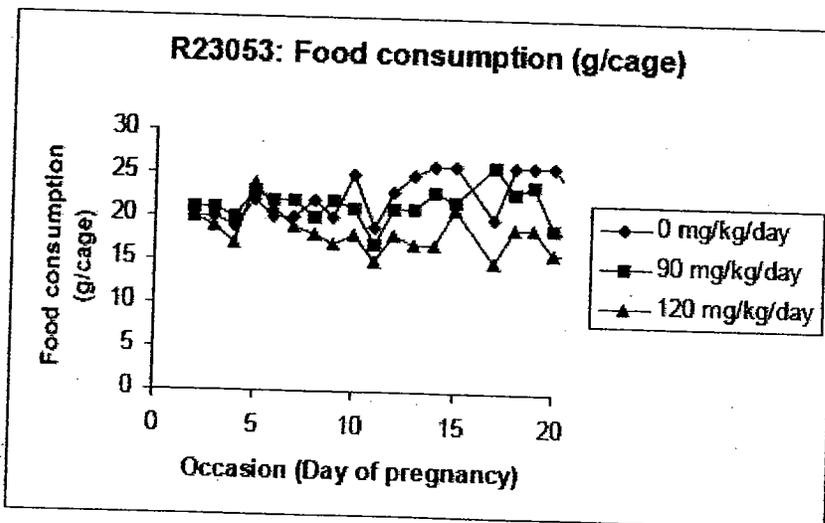
Body weight (dams):

The sponsor's graph below shows that the HD of 120 mg/kg led to a decrease in body weight gain in the dams treated with this dose.



Food consumption (dams):

The food consumption data, like the body weights, show that the HD rats were adversely affected by GW572016 treatment, with a slight decrease in food consumption seen. This is seen in the sponsor's graph, presented below.



Toxicokinetics:

The sponsor's table below shows that detectable levels of GW572016 were present in all the drug-treated rats after the first dose of drug and after the eleventh dose of drug. These data show that the plasma concentrations also increased with the increasing dose of GW572016 on the first day of dosing, but decreased on the eleventh day of dosing. However the standard deviation was very high for the LD on Day 17 and given that this is one time point measured on each day, there is not enough information to determine exactly why the plasma level is lower with the higher dose after repeated administration.

Table iii Average Plasma Concentrations of GW572016X in Pregnant Female Rats at 8-Hours After Single and Repeat Oral Administration of 0, 90 or 120mg GW572016X/kg/day

| Day | Dose (mg/kg/day) | Average (ng/mL) | SD (ng/mL) |
|----------------------------|------------------|-----------------|------------|
| 7 (1 st Dose) | 0 | BQL | BQL |
| | 90 | 17433 | 5414 |
| | 120 | 19174 | 2136 |
| 17 (11 th Dose) | 0 | BQL | BQL |
| | 90 | 22845 | 15174 |
| | 120 | 18099 | 4508 |

BQL = Below the Quantitation Limit (10 ng/mL)
SD = Standard Deviation

Terminal and necroscopic evaluations:C-section data:

Results of the uterine examinations are presented in the table below. The maternal GW572016 administration did not adversely affect the parameters measured.

| | Control | Low Dose 90 mg/kg | High Dose 120 mg/kg |
|--------------------------------|---------|----------------------|------------------------|
| Number corpora lutea | 12.0 | 13.0 | 12.0 |
| Number implantations | 11.3 | 13.0 | 10.7 |
| Percent pre-implantation loss | 5.6 | 0 | 11.1 |
| Intrauterine deaths | | | |
| Early | 0.0 | 0.0 | 0.3 |
| Late | 0.0 | 0.0 | 0.0 |
| Dead | 0.0 | 0.0 | 0.0 |
| Total | 0.0 | 0.0 | 0.3 |
| Percent post-implantation loss | 0 | 0 | 3.1 |
| Number live fetuses – mean/♀ | 11.3 | 13.0 | 10.3 |
| Live birth index (%) | 100 | 100 | 96.9 |
| Mean fetal weight (g) | 3.7 | 3.5 | 3.3 |

Offspring:

Not examined for malformations

Study title: GW572016F (ErbB2 inhibitor): Oral embryofetal development study in the pregnant Wistar Han rat.**Key study findings:**

- Slight decreases in body weight gain and food consumption in the HD dams
- No drug effects on the caesarian sectioning data
- No drug-related malformations seen in the litters
- Increased incidence of precocious ossification and developmental disturbances such as the presence of left umbilical artery and cervical rib

Study no.: WD/2001/00237/00
Volume #, and page #: Module 4.2.3.5.2.3
Conducting laboratory and location: Glaxo Wellcome Research and Development
Ware, Hertfordshire, UK
Date of study initiation: 17 October 2000
GLP compliance: Letter included and signed
QA reports: yes (X) no ()
Drug, lot #, and % purity: GW572016F, Lot# U14572/31/5, — purity

Methods

Doses: 30, 60 or 120 mg/kg/day
Species/strain: Rat/Wistar Han
Number/sex/group: 24 females/dose
Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose
+ 0.1% Tween 80, 10 mL/kg volume
Satellite groups used for toxicokinetics: 4 females/dose for toxicokinetics
Study design: F0 females dosed from GD 7-17, with the
day of mating noted as GD 1. Females then
ethanized on GD 21.
Parameters and endpoints evaluated: Females: in-life observations, body weight,
food consumption, gross necropsy, uterus
weight, corpora lutea, implantations,
resorptions, gross placental morphology,
live and dead fetuses, fetal weights,
toxicokinetics
F1 rats: fetal examinations (malformations
and alterations)

ResultsMortality (dams):

No maternal deaths during the study

Clinical signs (dams):

Clinical signs seen more frequently in the drug-treated groups included piloerection. One MD dam had a red-vaginal discharge on GD 14, but that resolved by the end of that day.

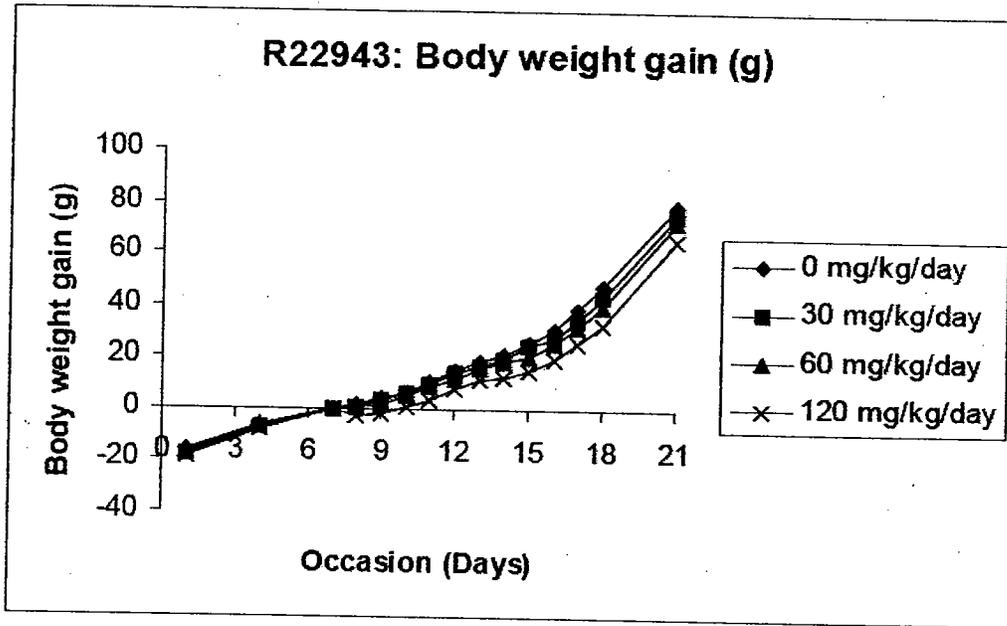
Body weight (dams):

Changes in body weights were seen during the treatment period. The table below shows the changes in body weight gains during several periods of gestation and the percent change from control seen in the high dose group. During the period of gestation when drug administration starts until two days before cessation, the HD dams gained 31% less weight than the control ones.

| Change in Body Weights Over Specified Period of Gestation | | | | | |
|---|---------|----------------------|----------------------|------------------------|--------------------------------------|
| Period of Gestation | Control | Low Dose 30 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg | Percent change HD from Control |
| GD 1 - 7 | 16.4 | 17.6 | 16.7 | 18.7 | + 14 % |
| GD 7 - 18 | 47.5 | 43.4 | 39.6 | 32.9 | - 30.7 % |
| GD 18 - 21 | 31.6 | 31.8 | 33.0 | 32.9 | + 4.1 % |

**APPEARS THIS WAY
ON ORIGINAL**

The sponsor's graph below shows that the HD of 120 mg/kg led to a slight decrease in body weight gain in the dams treated with this dose.



**APPEARS THIS WAY
ON ORIGINAL**

Food consumption (dams):

The food consumption data, presented in the sponsor's graph below, show that the HD rats had decreased food consumption during drug treatment. When the average food consumption over the period of drug treatment, GD7-18, was examined, both MD and HD rats ate significantly less food than the control rats. This is shown in the sponsor's table presented after the graph.

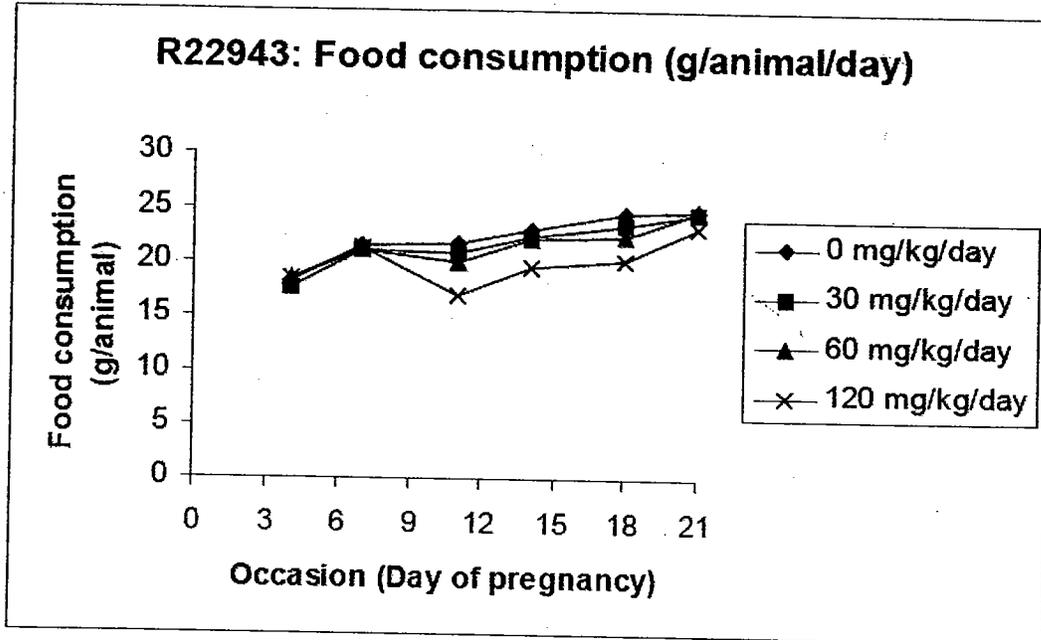


Table viii Group Mean Food Consumption During Pregnancy (g/animal/day)

| Period | Dose (mg/kg/day) | | | |
|----------|------------------|--------|---------|---------|
| | 0 | 30 | 60 | 120 |
| 1 to 7 | 19.864 | 19.295 | 19.761 | 19.909 |
| 7 to 18 | 23.079 | 22.248 | 21.494* | 18.905* |
| 18 to 21 | 24.652 | 24.455 | 24.826 | 23.227 |

*p<0.05

Toxicokinetics:

The sponsor's table below shows the pharmacokinetics of GW572016 in the pregnant rats, with samples taken on GD 7 and 17 at time points of pre-dose, 2, 4, 8, 12 and 24 hours after the drug administration. AUC and C_{max} increase with increasing doses of GW572016 and the data show that drug is not accumulating over the period of administration.

Table v Serum Concentrations of GW572016X in Pregnant Female Rats after Single and Repeat Oral Administration of 30, 60 or 120mg GW572016X/kg/day.

| Dose (mg/kg/day) | Pregnancy Day | AUC ¹ (h*ng/mL) | C_{max} (ng/mL) | T_{max} (h) | $t_{1/2}$ (h) |
|------------------|---------------|----------------------------|-------------------|---------------|---------------|
| 30 | 7 | 33013 | 4197 | 4 | 3.25 |
| | 17 | 48941 | 5540 | 4 | 3.50 |
| 60 | 7 | 153029 | 12581 | 4 | 3.77 |
| | 17 | 149729 | 17423 | 4 | 4.50 |
| 120 | 7 | 241519 | 26312 | 2 | 7.21 |
| | 17 | 294641 | 21401 | 2 | 9.02 |

n = 12 rats per dose per day (two rats per timepoint)

1. AUC_{0-24h} on Day 7 of pregnancy (first day of dosing) and AUC_{24h} on Day 17 of pregnancy (11th day of dosing).

Terminal and necroscopic evaluations:C-section data:

Results of the uterine examinations are presented in the table below. The maternal GW572016 administration did not adversely affect the parameters measured. The HD dams had an increase in early post-implantation loss, but this was within background for this species of rat. There was also one HD dam that had total embryofetal loss, though the litter was only one pup that was an early intra-uterine death.

| | Control | Low Dose 30 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|--------------------------------|---------|----------------------|----------------------|------------------------|
| Number corpora lutea | 11.8 | 11.5 | 11.7 | 11.8 |
| Number implantations | 11.0 | 10.6 | 10.3 | 11.6 |
| Percent pre-implantation loss | 7.0 | 7.5 | 11.6 | 5.5 |
| Post-implantation loss | | | | |
| Early | 0.3 | 0.6 | 0.3 | 0.7 |
| Late | 0.0 | 0.0 | 0.0 | 0.0 |
| Dead | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 0.3 | 0.6 | 0.3 | 0.7 |
| Percent post-implantation loss | 2.8 | 6.0 | 3.4 | 6.3 |
| Number live fetuses – mean/♀ | 10.7 | 10.0 | 10.0 | 10.9 |
| Live birth index (%) | 97.2 | 94.0 | 96.6 | 93.8 |
| Mean fetal weight (g) | 3.6 | 3.7 | 3.7 | 3.5 |

Offspring:***Malformations***

Malformations were seen in litters of all dose groups, with 2 litters (4 pups) in the control group and 1 litter (1 pup) each in the GW572016 treated groups. The malformations are listed below:

Control:

- Maxillary process/jugal fusion, unilateral, severe
- Maxillary process/jugal fusion, unilateral, severe
- Microphthalmia, unilateral; absent azygous vein
- Interrupted posterior vena cava, left adrenal gland displaced by continuous azygous vein

LD:

- Scapula bent, bilateral, severe; humerus shortened and thickened, bilateral, severe

MD:

- Absent azygous vein

HD:

- Maxillary process/jugal fusion, unilateral, slight

These results indicate that under the design of this study, GW572016 did not increase the incidence of malformations in the offspring that were exposed prenatally to the drug during organogenesis.

Alterations

GW572016 exposure during organogenesis increased the incidence of several developmental disturbances and precocious ossification. The sponsor's table below shows the increases in the persistence of the left umbilical artery, an artery present during gestation that normally regresses in the last 10 days of gestation, while the right umbilical artery persists. As this process, normal in rat development, is not seen in the humans where both umbilical arteries remain throughout gestation, its relevance to human development is not known but it is likely not relevant. The table also shows the presence of cervical rib, an extra rib or pair of ribs associated with the 7th cervical vertebra. This occurs spontaneously in around 5% of fetuses of this rat strain and this study shows that the incidence is higher than that in all GW572016 exposed litters. Published literature (Leffert RD and Perlmutter GS, 1999) indicates that this alteration clinically may be associated with compression of the nerves and associated blood vessels of the brachial plexus, also known as thoracic outlet syndrome.

| Parameter | Dose (mg/kg/day) | | | | Background incidence mean (range) |
|---|------------------|------|------|------|-----------------------------------|
| | 0 | 30 | 60 | 120 | |
| Left umbilical artery | | | | | |
| % fetuses affected | 11.8 | 12.3 | 11.4 | 16.7 | 10.5 (8.7 - 13.1) |
| % litters affected | 69.6 | 68.2 | 56.5 | 86.4 | 61.2 (47.8 - 73.9) |
| Cervical rib (on 7th cervical vertebra) - uni/bilateral, rudimentary/short/long | | | | | |
| % fetuses affected | 3.9 | 7.9 | 7.6 | 9.4 | 5.2 (2.9 - 8.4) |
| % litters affected | 17.4 | 27.3 | 30.4 | 31.8 | 20.9 (13.0 - 34.8) |

Precocious ossification is also seen in this study, evident by decreases in incidences of “incompletely ossified” structures and increases in incidences of “ossified” structures. The sponsor’s table below shows 4 bones with decreases in incomplete ossification and increases in the cervical vertebral centra with precocious ossification. These effects are seen in all the dose groups, with statistical significance seen mostly at the HD level.

| % fetuses affected | Dose (mg/kg/day) | | | | Background incidence mean (range) |
|--|------------------|------|--------|--------|-----------------------------------|
| | 0 | 30 | 60 | 120 | |
| Frontal, unilateral and bilateral | 13.3 | 7.0 | 6.7 | 0.8** | 15.4 (6.7 - 21.6) |
| Parietal, bilateral | 39.1 | 27.2 | 27.7 | 20.5** | 42.7 (27.4 - 51.9) |
| Squamosal, unilateral and bilateral | 25.8 | 16.7 | 17.6 | 11.0** | 21.6 (9.4 - 34.2) |
| Squamosal process, bilateral | 8.6 | 6.1 | 4.2 | 2.4 | 5.8 (1.9 - 12.6) |
| All cervical vertebral centra ossified | 7.0 | 8.8 | 21.8** | 13.4 | 7.8 (0.8 - 19.2) |

** > p 0.01

Study title: GW572016F: Non-audited dose-range finding toxicity study in non-pregnant New Zealand white rabbits.

Key study findings:

- Doses tested relatively well tolerated
- HD had some clinical observation effects (loose feces), decreased food consumption and body weight, all noted in 1/3 of the rabbits at this dose

Study no.: RD/2000/00574/00
Volume #, and page #: Module 4.2.3.5.2.4
Conducting laboratory and location: Glaxo Wellcome Inc.
Medicines Safety Evaluation Division
Five Moore Drive
Research Triangle Park, NC
Date of study initiation: 26 April 2000
GLP compliance: No
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016F, Lot# U14572/36/3, — . purity

Methods

Doses: 30, 60, 120 and 180 mg/kg/day
Species/strain: Rabbit/New Zealand white
Number/sex/group: 3 females/dose
Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 5 mL/kg volume
Satellite groups used for toxicokinetics: None – blood taken from main rabbits on Day 1 and Day 14 at time points pre-dose, 1, 2, 4, and 8 hours after
Study design: Non-pregnant rabbits were dosed for 14 days, once daily
Parameters and endpoints evaluated: In-life observations, body weight, food consumption, gross necropsy, toxicokinetics

Results

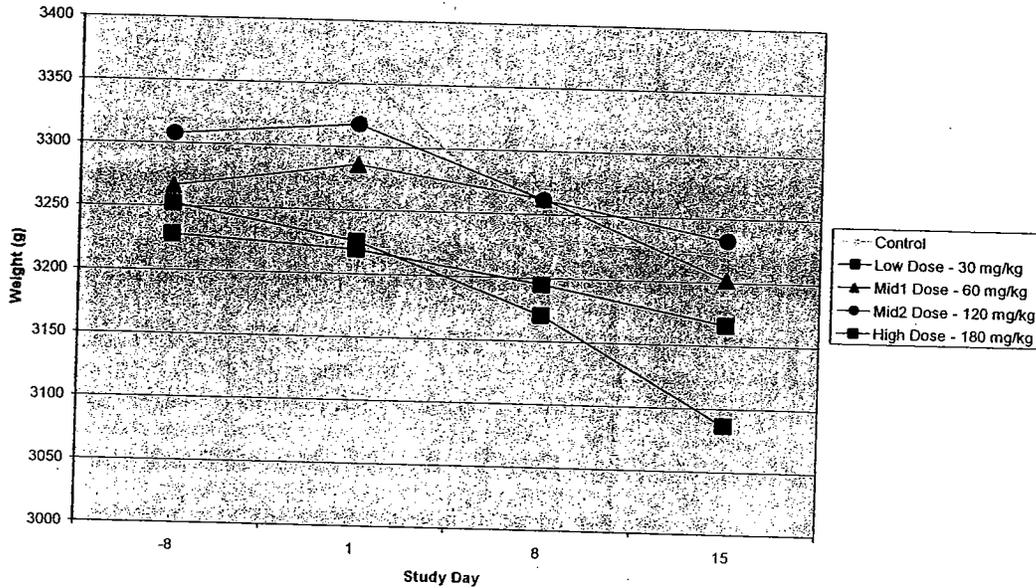
Mortality:
No mortality

Clinical signs:
Loose feces and reduced eating were seen in 1/3 HD rabbits toward the end of the dosing regimen

Body weight:

The graph below shows the body weights of the rabbits treated with either the vehicle or 3 doses of GW572016. A slight decrease in body weight was seen at the HD by the end of the treatment period, due primarily to one HD rabbit.

**Non-Pregnant Rabbit Body Weights
During Treatment with GW572016**

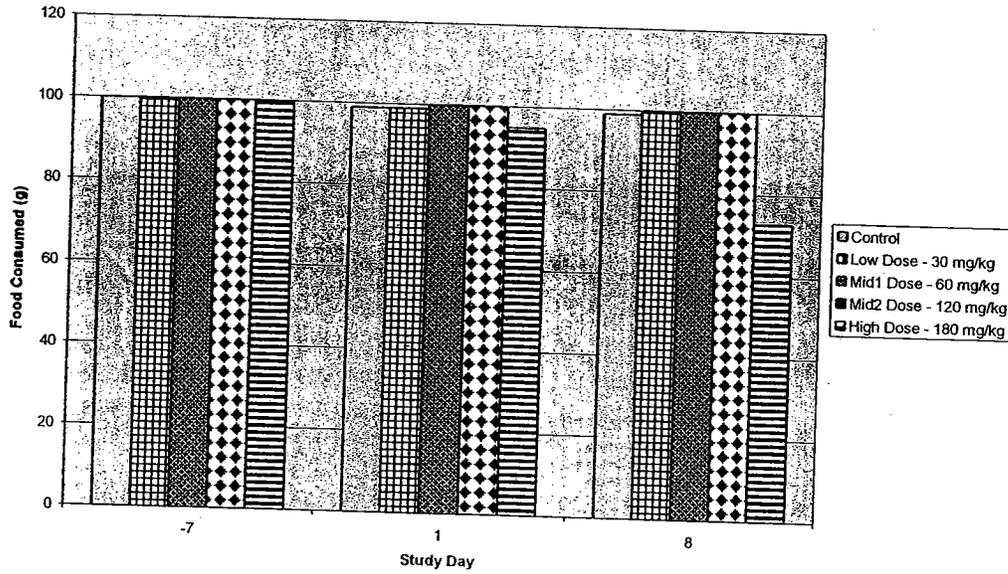


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Food consumption:

The food consumption data is shown in the graph below, with decreased food eaten by the HD rabbits, due to one rabbit's decreased eating, the same rabbit that is responsible for the decrease in mean body weight seen in the HD group.

**Food Consumption in Non-Pregnant Rabbits
Treated With GW572016**



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Toxicokinetics:

The sponsor's table below lists the toxicokinetic results from this study. The C_{max} increases in a dose-proportional manner as the GW572016 dose increases, as do all the AUC points other than the Day 14 time point for the HD group. The drug half-life is not affected by the increasing dose of GW572016.

| Dose (mg/kg/day) | Day | | AUC ¹ (h*ng/mL) | C _{max} (ng/mL) | T _{max} (h) | t _{1/2} (h) | Dose-Normalized | |
|------------------|-----|------|----------------------------|--------------------------|----------------------|----------------------|----------------------------|--------------------------|
| | | | | | | | AUC ¹ (h*ng/mL) | C _{max} (ng/mL) |
| 30 | 1 | Mean | 477 | 122 | 1-4 | 1.69 | 477 | 122 |
| | | SD | 121 | 52.2 | | 0.33 | 121 | 52.2 |
| | 14 | Mean | 520 | 166 | 1-2 | 2.34 | 520 | 166 |
| | | SD | 35.3 | 47.8 | | 0.42 | 35.3 | 47.8 |
| 60 | 1 | Mean | 886 | 219 | 1-2 | 1.59 | 443 | 110 |
| | | SD | 225 | 51.2 | | 0.20 | 113 | 25.6 |
| | 14 | Mean | 768 | 209 | 2 | 2.37 | 384 | 104 |
| | | SD | 318 | 100 | | 0.90 | 159 | 50.1 |
| 120 | 1 | Mean | 2129 | 426 | 1 | 2.66 | 532 | 106 |
| | | SD | 75.3 | 147 | | 0.68 | 18.8 | 36.7 |
| | 14 | Mean | 2079 | 414 | 2 | 2.50 | 520 | 104 |
| | | SD | 1101 | 185 | | 0.90 | 275 | 46.4 |
| 180 | 1 | Mean | 3088 | 668 | 1-2 | 2.06 | 515 | 111 |
| | | SD | 873 | 179 | | 0.30 | 146 | 29.9 |
| | 14 | Mean | 5489 | 1112 | 2-8 | 1.79 | 915 | 185 |
| | | SD | 4079 | 882 | | 0.97 | 680 | 147 |

1. AUC₀₋₂₄ on Day 1 and AUC₀₋₂₄ on Day 14

n = 3 females per dose group except Day 14 for 30 mg/kg/day (n = 2)

Terminal and necropsic evaluations: C-section data:

Non-pregnant rabbits studied to determine a maximum dose for use in pregnant rabbits. No macroscopic effects of GW572016 administration were seen in these rabbits.

Offspring:

No offspring in this study

Study title: GW572016F (ErbB2 inhibitor): Study to determine the maximum repeatable daily oral dose in the New Zealand white rabbit.

Key study findings:

- The dose of 400 mg/kg was too toxic for use in a reproductive study, as the rabbits were euthanized moribund and eating little to no food during the drug administration

Study no.: WD/2000/00414/00
Volume #, and page #: Module 4.2.3.5.2.5
Conducting laboratory and location: Glaxo Wellcome Research and Development
Ware, Hertfordshire, UK
Date of study initiation: 28 July 2000
GLP compliance: No
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016F, Lot# U14572/31/5, — purity

Methods

Doses: 400 mg/kg/day
Species/strain: Rabbit/New Zealand white
Number/sex/group: 3 females/dose
Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose
+ 0.1% Tween 80, 5 mL/kg volume
Satellite groups used for toxicokinetics: None – blood taken from main rabbits on
Day 1 and Day 7 at time points pre-dose,
0.5, 1, 2, 4, 8 and 12 hours after dosing on
Day 1 and at 3 hrs post dosing on Day 7 due
to euthanizing moribund animals
Study design: Non-pregnant rabbits were dosed for 7 days,
once daily
Parameters and endpoints evaluated: In-life observations, body weight, food
consumption, gross necropsy, toxicokinetics

Results

Mortality:

All 3 rabbits were euthanized moribund on Day 7

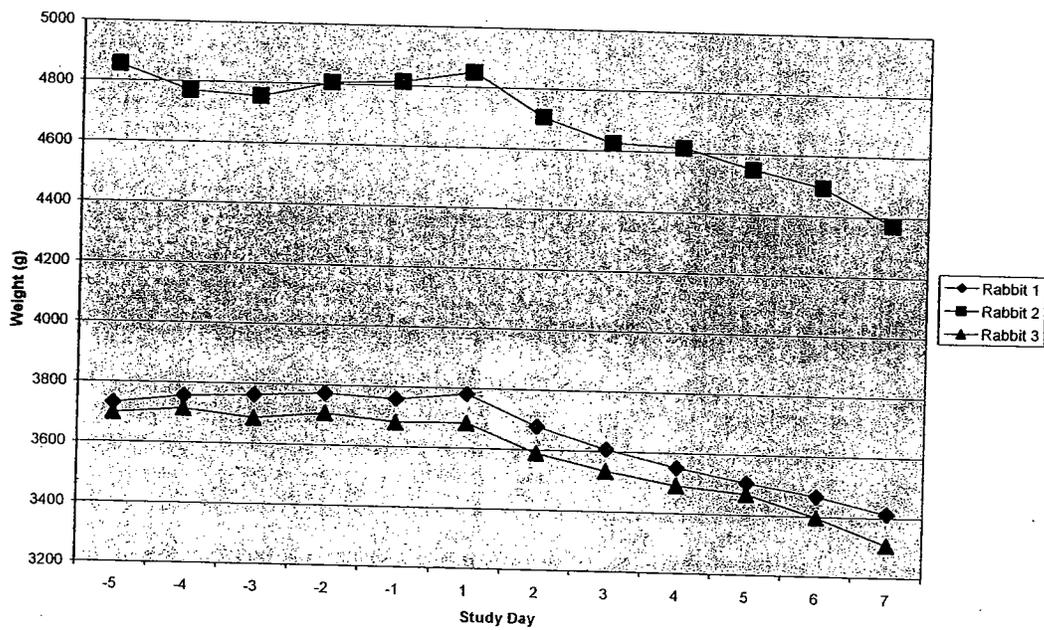
Clinical signs:

Reduced fecal output was seen in all 3 of the rabbits starting on Day 2 of dosing

Body weight:

The graph below shows the body weights of the three rabbits, with decreases in body weight seen in all three rabbits during the 7 days of drug administration.

Rabbit Weights Before and During Treatment with GW572016

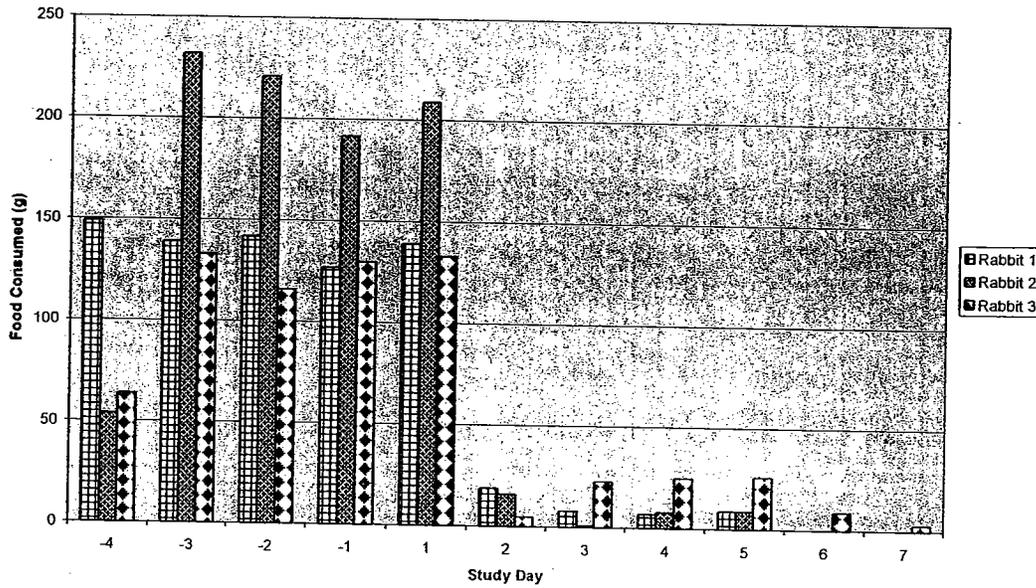


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Food consumption:

The food consumption data, shown in the graph below, shows the severe effect that treatment with 400 mg/kg/day had on the rabbit's eating, beginning from the second day of dosing.

Food Consumption Before and During Treatment With GW572016



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Toxicokinetics:

The sponsor's table below shows that detectable levels of GW572016 were present in the non-pregnant rabbits. When the toxicokinetics for this dose are compared with the data obtained in the rabbit toxicology study with 30, 60, 120 and 180 mg/kg, the C_{max} is shown to increase in a dose-proportional fashion, but the AUC increased in a greater than proportional manner. Half-life also increased with this dose over the doses previously studied in the rabbit.

Table 2. Toxicokinetic Parameters of GW572016X in Female Non-Pregnant Rabbits Following Single Oral Administration of GW572016F at 400 mg/kg/day GW572016X

| Animal Number | AUC ¹ (h*ng/mL) | C_{max} (ng/mL) | T_{max} (h) | $t_{1/2}$ (h) | Dose-Normalized | |
|---------------|-------------------------------|----------------------|------------------|------------------|------------------------|---------------------------------|
| | | | | | C_{max}^2 (ng/mL) | AUC ^{1,2} (h*ng/mL) |
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| Mean | 33629 | 2029 | 2.4 | 9.53 | 152 | 2522 |
| SD | 23419 | 988 | | 2.69 | 74.1 | 1756 |

¹ The area under the curve (AUC) is the AUC_∞.

² The AUC and C_{max} values were normalized to 30 mg/kg/day from Report RD2000/00574/00.
SD = Standard Deviation

Terminal and necropsic evaluations:C-section data:

Non-pregnant rabbits studied to determine a maximum dose for use in pregnant rabbits. No macroscopic effects of GW572016 administration were seen in these rabbits.

Offspring:

No offspring in this study

Study title: GW572016F (ErbB2 inhibitor): Maximum repeatable dose study in the pregnant New Zealand white rabbit.

Key study findings:

- MD2 and HD groups terminated early, due to decreased food consumption
- MD1 dose of 120 mg/kg considered the maximum repeatable dose in pregnant rabbits due to slight reduction in food consumption at this dose and the fact that the dose of 200 mg/kg was too toxic

Study no.: WD/2000/00520/00
Volume #, and page #: Module 4.2.3.5.2.6
Conducting laboratory and location: Glaxo Wellcome Research and Development
 Ware, Hertfordshire, UK
Date of study initiation: 30 August 2000
GLP compliance: No
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016F, Lot# U14572/31/5, — , purity

Methods

Doses: 90, 120, 200, or 300 mg/kg/day
Species/strain: Rabbit/New Zealand white
Number/sex/group: 3 females/dose
Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 5 mL/kg volume
Satellite groups used for toxicokinetics: None – blood taken from main rabbits on GD 8 and GD 20 at time points pre-dose, 0.5, 1, 2, 4, 8 and 12 hours after dosing
Study design: Pregnant rabbits were dosed on GD 8 – 20 inclusive and then euthanized and C-sectioned on GD 21
Parameters and endpoints evaluated: In-life observations, body weight, food consumption, gross necropsy, uterine examination (corpora lutea, implantations, resorptions, live fetuses, post-implantation loss), toxicokinetics

Results

Mortality:

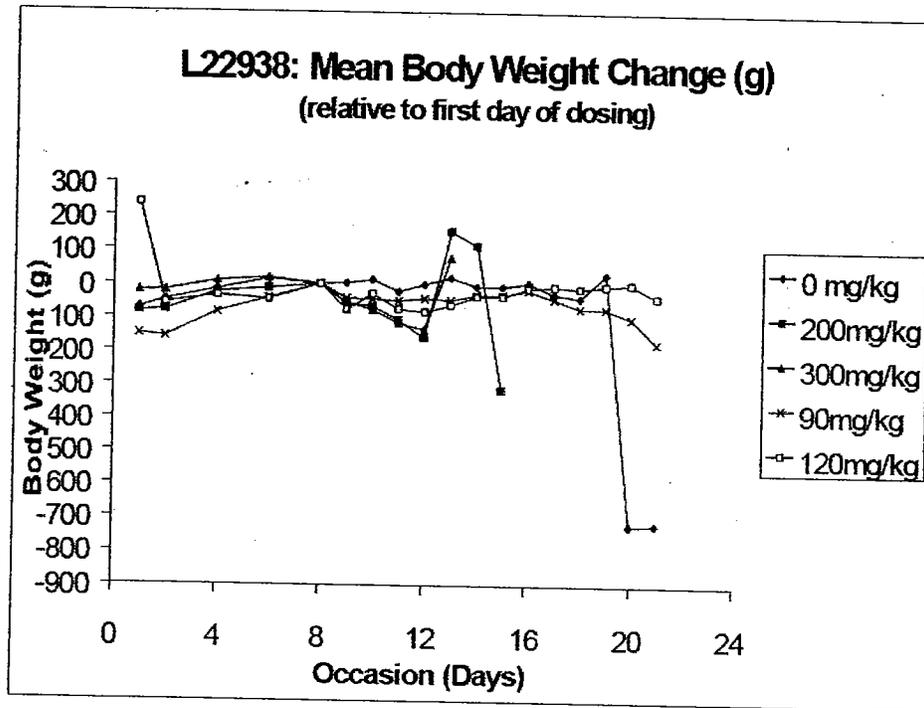
All rabbits in the HD and MD2 group were euthanized moribund between GD11 - GD 15. This was due primarily to the decreased food consumption and an accompanying slight decrease in body weight gain. There was also one control rabbit that was not eating, had a soiled/swollen anogenital area and was euthanized on GD 19. Another control rabbit was accidentally killed on GD 18 and a MD1 rabbit was found dead on GD 2, before dosing even began.

Clinical signs:

Reduced fecal output was seen in all rabbits treated with the LD and MD1 doses, and was also noted in the animals that were euthanized moribund.

Body weight:

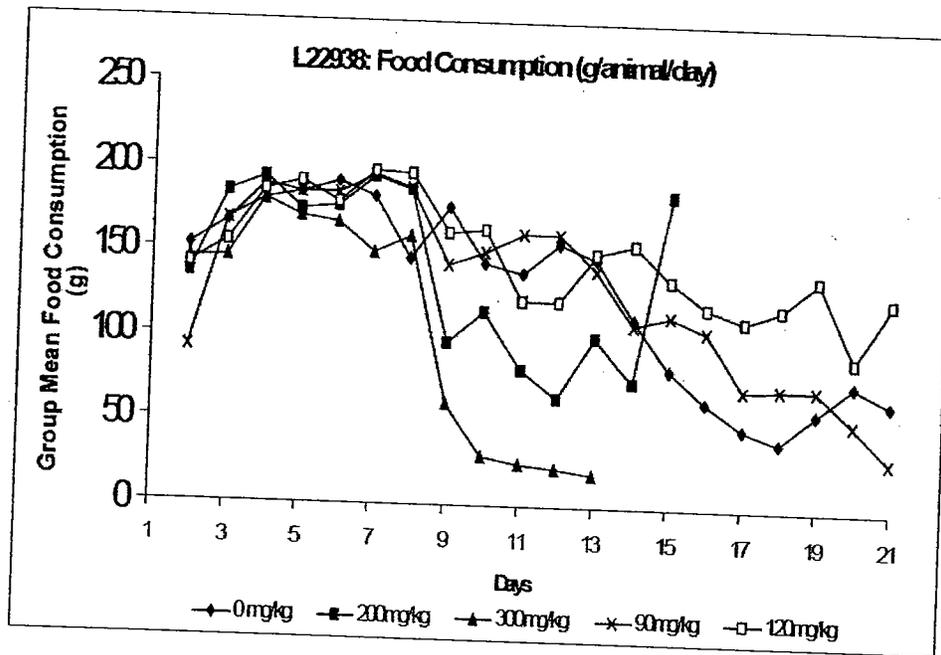
The sponsor's graph below shows the body weight changes, relative to the first day of dosing, in all the rabbits, including the ones that did not survive until the conclusion of the study. In the rabbits that survived to the end of dosing, body weight changes were not affected by drug treatment.



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Food consumption:

The sponsor's graph below shows the food consumption in all dose groups. The drastic decreases in food consumption in the two dose groups (MD2 and HD) terminated early can be seen.



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Toxicokinetics:

The sponsor's table below shows that detectable levels of GW572016 were present in all treatment rabbits. Data were obtained for the LD and MD1 groups on GD 8 and 20, but only on GD 8 for the MD2 and HD groups due to the premature sacrifice of these dose groups. While the parameters increase primarily in a dose-response manner for the LD and MD1 groups, the limited data from the MD2 and HD groups show saturation occurring, as these are increasing in a less than proportional fashion.

| Dose (mg/kg/day) | Day | | AUC ¹ (h*ng/mL) | C _{max} (ng/mL) | T _{max} (h) | t _{1/2} (h) | Dose-Normalized | |
|---------------------|-----|------|-------------------------------|-----------------------------|-------------------------|-------------------------|-------------------------------|-----------------------------|
| | | | | | | | AUC ¹ (h*ng/mL) | C _{max} (ng/mL) |
| 90 | 8 | Mean | 8556 | 1042 | 2 | 4.07 | 8556 | 1042 |
| | | SD | 2601 | 348 | | 0.32 | 2601 | 348 |
| | 20 | Mean | 9036 | 493 | 4 | 32.0 | 9036 | 493 |
| | | SD | 6009 | 246 | | 39.0 | 6009 | 246 |
| 120 | 8 | Mean | 11385 | 1228 | 2 | 5.02 | 8538 | 921 |
| | 20 | Mean | 8325 | 816 | 4.8 | 3.57 | 6244 | 612 |
| 200 | 8 | Mean | 13400 | 1556 | 2.4 | 5.26 | 6030 | 700 |
| | | SD | 2715 | 226 | | 3.18 | 1222 | 102 |
| 300 | 8 | Mean | 17569 | 1271 | 2.4 | 7.48 | 5271 | 381 |
| | | SD | 11410 | 367 | | 3.48 | 3423 | 110 |

n = 3 females except for the 120 mg/kg/day dose group (n=2).
 AUC_∞ on Day 8 (1st dose) and AUC_{24h} on Day 20 (13th dose)

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ON ORIGINAL**

Terminal and necroscopic evaluations:C-section data:

The table below presents the uterine parameters with the control group and two GW572016 dose groups that survived to the end of the study. There was no significant drug effect on any of these parameters. Although it appears that GW572016 administration leads to a decrease in implantations and number of live fetuses, and an increase in pre-implantation loss, there is no dose relationship and the values in the control group were not within the historical ranges.

| | Control | Low Dose 90 mg/kg | Mid1 Dose 120 mg/kg |
|--------------------------------|---------|----------------------|------------------------|
| Number corpora lutea | 11.0 | 10.0 | 10.0 |
| Number implantations | 11.0 | 7.0 | 9.0 |
| Percent pre-implantation loss | 3.2 | 30.0 | 10.0 |
| Intrauterine deaths | | | |
| Early | 0.0 | 0.3 | 0.5 |
| Late | 0.0 | 0.0 | 0.0 |
| Dead | 0.0 | 0.0 | 0.0 |
| Total | 0.0 | 0.3 | 0.5 |
| Percent post-implantation loss | 2.7 | 4.9 | 5.6 |
| Number live fetuses – mean/♀ | 10.0 | 6.7 | 8.5 |
| Live birth index (%) | 100 | 95.2 | 94.4 |

Macroscopic changes noted in the rabbits that were euthanized were primarily limited to stomach findings and likely due to the lack of food consumption.

Offspring:

Offspring not examined in this study

**APPEARS THIS WAY
ON ORIGINAL**

Study title: GW572016F: Oral embryo-fetal development study in New Zealand white rabbits.

Key study findings:

- HD led to significant decrease in body weights and food consumption as well as one maternal death and four abortions, though the body weight effect was not a sign of maternal toxicity as the corrected body weights do not differ significantly
- MD also led to decreased body weights and significantly decreased food consumption
- No significant drug effect on malformations
- Decreased fetal body weights and increased number of fetuses and litters with skeletal variations at the MD and HD

Study no.:

RD/2001/00010/00

Volume #, and page #:

Module 4.2.3.5.2.7

Conducting laboratory and location:

Date of study initiation:

24 December 2000

GLP compliance:

Letter included and signed

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

GW572016F, Lot# R5361/44/1. — .purity

Methods

Doses:

30, 60, and 120 mg/kg/day

Species/strain:

Rabbit/New Zealand white

Number/sex/group:

20 females/dose

Route, formulation, volume, infusion rate:

PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 5 mL/kg volume

Satellite groups used for toxicokinetics:

5 rabbits/dose for toxicokinetics, blood taken on GD 7 and 20 with time points of pre-dose, 0.5, 1, 2, 4, 8, and 12 hrs after dosing

Study design:

Pregnant rabbits were dosed on GD 7 – 20 inclusive with the day of mating identified as GD0. rabbits were euthanized and C-sectioned on GD 29

Parameters and endpoints evaluated:

In-life observations, body weight, food consumption, gross necropsy, uterine examination (corpora lutea, implantations, resorptions, live fetuses, post-implantation loss), fetal examinations (external, visceral, skeletal), toxicokinetics

Results

Mortality:

One HD rabbit was found dead on GD 19 after 12 doses of GW572016. The animal showed signs of emaciation, unkempt coat, liquid or soft feces and had lost weight with reduced food consumption. Upon necropsy it was noted that the doe had 8 early resorptions *in utero*.

Clinical signs:

Clinical signs for the animal found dead and the 4 rabbits euthanized after aborting all or part of their litters are presented elsewhere in this review. In general, clinical signs associated with GW572016 administration were seen in the MD and HD groups and included:

- Soft or liquid feces
- Scant feces
- Alopecia
- Ungroomed coat

Abortions:

Four HD rabbits aborted their litters while on study. The table below shows the details of the 4 HD rabbits. While aborting fetuses is not uncommon in laboratory rabbits, there were only HD rabbits with abortions, so it would seem to clearly be an effect of GW572016 administration during gestation.

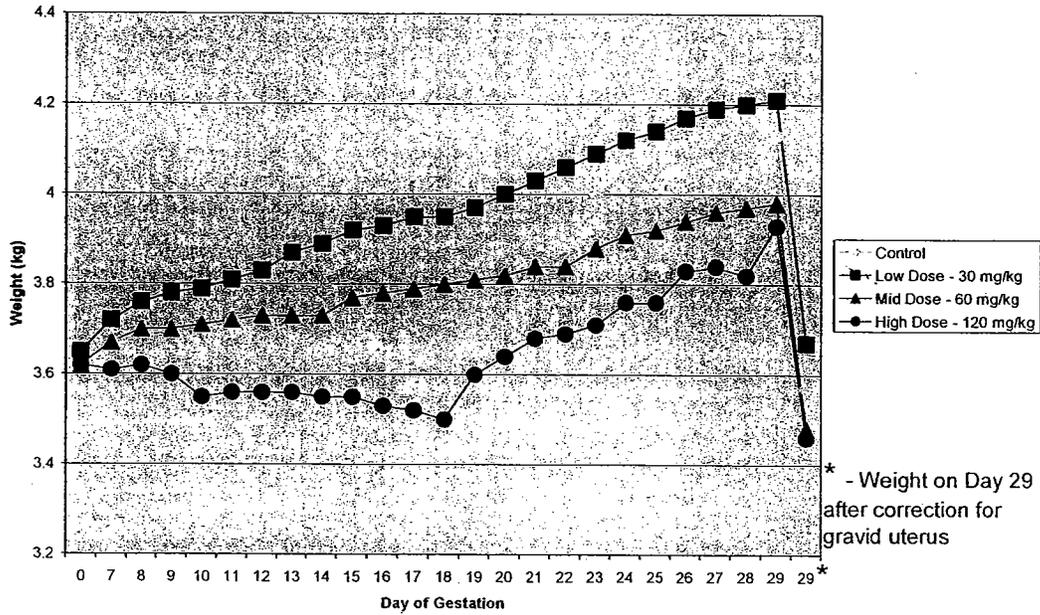
| Rabbit # | Day aborted | | Clinical and gross observations |
|-------------|-------------|---|---|
| Rabbit 4970 | GD 19 | 5 late resorption 7 early resorptions | Scant feces, ↓ weight and food consumption, numerous green areas on liver |
| Rabbit 4972 | GD 25 | 10 live fetuses 2 late resorptions | Red vaginal discharge, loose stool or scant feces, ↓ weight and food consumption, normal gross necropsy |
| Rabbit 4973 | GD 28 | 6 dead fetuses 2 late resorptions | Loose stool or scant feces, red substance in cage pan, ↓ weight and food consumption, normal gross necropsy |
| Rabbit 4974 | GD 20 | 8 late resorptions 2 early resorptions | Loose stool, emaciation, red substance in cage pan, ↓ weight and food consumption, green medulla of kidneys, green lobes of liver, pale lungs |

Body weight:

The graph below shows the body weights of the pregnant does throughout gestation. From GD 14 until GD 28 the weights of the HD rabbits were significantly lower than the

weights of the control rabbits. When the body weights on Day 29 were corrected for the gravid uterus, the HD rabbits were not significantly different from controls, indicating that the significant differences seen were due to the uterine contents and not to the actual maternal body weights.

Maternal Rabbit Weights During Gestation

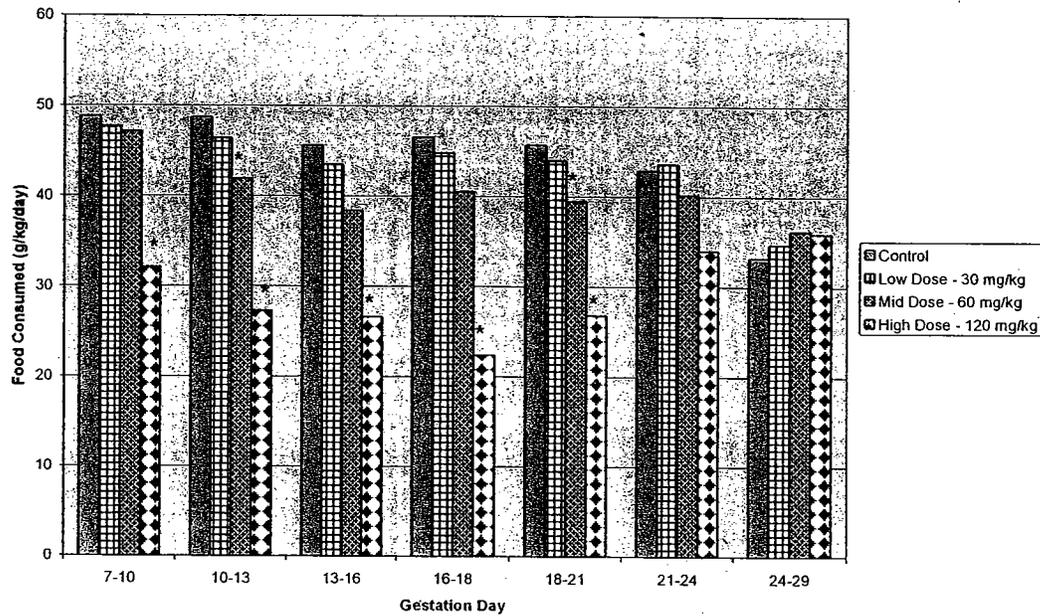


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Food consumption:

The graph below shows the food consumption, relative to the rabbits' body weights. Throughout drug administration, the HD rabbits ate significantly less food, both absolute amounts and relative to body weight, than did the control rabbits. MD rabbits also ate significantly less food at two time points during dosing. Food consumption in the HD group rebounded after dosing and both the MD and HD groups ate comparable amounts of food compared to control.

Maternal Relative Food Consumption During Gestation



**APPEARS THIS WAY
ON ORIGINAL**

Toxicokinetics:

The sponsor's table below shows that detectable levels of GW572016 were present in all treatment rabbits. Mean AUC and C_{max} values were not significantly different in each dose group between Days 7 and 20, indicative of no significant drug accumulation. When the AUC and C_{max} were dose-normalized to the LD level, the increases were greater than dose-proportional for the MD and HD groups. Half-life values were not statistically different across dose groups.

| Mean Serum Toxicokinetic Parameters of GW572016X in Pregnant Rabbits | | | | | | | | |
|--|------------------|------|-------------------------------|----------------------|------------------|------------------|-------------------------------|----------------------|
| Dose (mg/kg/day) | Pregnancy Day | | AUC ¹ (h*ng/mL) | C_{max} (ng/mL) | T_{max} (h) | $t_{1/2}$ (h) | Dose-Normalized ² | |
| | | | | | | | AUC ¹ (h*ng/mL) | C_{max} (ng/mL) |
| 30 | 7 | Mean | 825 | 116 | 1.4 | 3.60 | 825 | 116 |
| | | SD | 163 | 31.3 | | 2.51 | 163 | 31.3 |
| | 20 | Mean | 1032 | 144 | 1.4 | 2.35 | 1032 | 144 |
| | | SD | 307 | 45.5 | | 0.48 | 307 | 45.5 |
| 60 | 7 | Mean | 3115 | 464 | 4 | 2.20 | 1557 | 232 |
| | | SD | 1211 | 179 | | 0.53 | 606 | 89.5 |
| | 20 | Mean | 2943 | 418 | 1.4 | 2.65 | 1472 | 209 |
| | | SD | 1175 | 214 | | 0.64 | 587 | 107 |
| 120 | 7 | Mean | 3500 | 409 | 4.8 | 2.57 | 875 | 102 |
| | | SD | 1038 | 132 | | 0.53 | 260 | 32.9 |
| | 20 | Mean | 8453 | 667 | 4.8 | 3.67 | 2113 | 167 |
| | | SD | 5928 | 397 | | 1.01 | 1482 | 99.3 |

n= 5 per dose per day

1. AUC₀₋₂₄ on Day 7 and AUC₀₋₂₄ on Day 20.

2. The AUC and C_{max} values were normalized to 30 mg/kg/day.

Terminal and necropsic evaluations:

Necropsy results of the does showed an absent intermediate lung lobe in one LD rabbit and a small gall bladder in a control rabbit. Other necropsy results previously described in the rabbits that aborted and the rabbit found dead.

C-section data:

The table below presents the uterine parameters for the control and GW572016 dose groups. The average fetal weights were decreased in the MD and HD groups, reaching statistical significance in the HD group. No other parameters were affected by the drug treatment.

| | Control | Low Dose 30 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|---|---------|----------------------|----------------------|------------------------|
| Litters with one or more live fetus | 19 | 18 | 17 | 14 |
| Number implantations | 8.8 | 8.2 | 8.5 | 8.1 |
| Live fetuses | 8.6 | 8.0 | 8.1 | 7.6 |
| Percent live male fetuses/litter | 52.1 | 53.6 | 56.4 | 52.2 |
| Live fetal body weights/litter | | | | |
| Mean pup weights | 45.43 | 47.04 | 42.97 | 41.00* |
| Mean male weight | 45.95 | 47.46 | 42.86 | 42.10 |
| Mean female weights | 44.89 | 45.87 | 41.78 | 39.67* |
| Percent dead or resorbed conceptuses/litter | 2.2 | 2.5 | 5.6 | 5.2 |

Offspring:

Litters exposed *in utero* to GW572016 were compared to litters exposed to only the vehicle. Incidences of malformations (irreversible changes not common in this strain of rabbit) and variations (common findings in this strain and reversible delays or accelerations in development) were recorded for gross, soft tissue and skeletal alterations. The table below shows the incidence of any type of alteration, malformation or variation. There was a significant drug effect, as alterations increased, both number of pups and number of litters, with both the MD and HD groups when compared to control.

| | Control | Low Dose 30 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|---|----------|----------------------|----------------------|------------------------|
| Number of pups with any alterations (%) | 3 (1.8) | 11 (7.6) | 17 (12.3)* | 14 (13.1)* |
| Number of litters with at least one pup w/ any alterations (%) | 3 (15.8) | 6 (33.3) | 9 (52.9)* | 9 (64.3)* |
| Percent of fetuses with any alterations/litter | 1.6 | 7.1 | 11.0* | 13.6* |

Fetal gross alterations

Gross alterations were seen in three pups

- One HD pup with umbilical hernia
- Two Control pups with short tails – with fused caudal vertebrae

Fetal soft tissue alterations

Two pups had soft tissue **malformations**, as noted below:

- One HD pup with intestines protruding through umbilicus (confirmed umbilical hernia)
- One LD pup with absent ventricular semilunar valve, small right ventricle and distended aorta

Soft tissue **variations** included the following findings in the eye and lung:

- Two MD and one HD pups with circumcorneal hemorrhage
- Four fetuses from one MD litter with discolored lenses
- Absence of the intermediate lobe of lung in two fetuses from one LD litter, one fetus from one MD litter and two fetuses from two HD litters

It is unlikely that the discolored lens is due to GW572016 given the lack of any dose response and that only one litter was affected. Circumcorneal hemorrhage may be due to trauma during the processing and the absent intermediate lobe of lung has been noted in the literature to be common in this strain of rabbit.

Fetal skeletal alterations

Skeletal **malformations** were seen in the thoracic vertebrae/ribs and the lumbar vertebrae:

- One HD fetus with small right 10th thoracic arch, left 11th and 12th arches fuse, unilateral ossification of the left 10th arch and also with fused ribs (right 3rd and 4th proximal to distal and 11th and 12th fused distally)
- One MD fetus with a bifid 2nd centrum and fused 1st and 2nd centra
- Two LD fetuses with fused and/or small caudal vertebrae

Skeletal **variations** were seen in the skull, hyoid bone, ribs, sternum and in the fetal ossification site averages.

- Irregularities in ossification of the skull (incomplete ossification, presence of small ossification sites within the sutures of calvaria, and/or irregular shaping or fusion of the sutures or bones) in one control fetus (one litter), two LD fetuses (one litter), three MD fetuses (three litters) and three HD litters (two litters)
- Angulated ala(e) of the hyoid was seen in one control fetus (one litter), one LD fetus (one litter), two MD fetuses (two litters) and seven HD litters [significantly different from control] (three litters)
- Thickened rib in one fetus from one LD litter and in two MD fetuses from two litters
- Fused sternal centra from two control fetuses (two litters), three LD fetuses (three litters) and two MD fetuses (two litters)
- Significant decrease in the average number of forelimb phalange ossification sites in the HD group, though the incidence was still within historical range for the laboratory

Prenatal and postnatal development

Study title: GW572016F: Oral pre- and postnatal development study in rats.

Key study findings:

- HD was maternally toxic, decreased body weights and food consumption, though the effect on body weights were likely due to the
- Reduced food consumption in MD dams
- Decreased pup viability primarily in the early post natal days (0-4) in the HD and MD litters
- Evidence of exposure to GW572016 in the pup serum on PND 10, perhaps via maternal milk
- Decreased pre-weaning body weights in the F1 HD pups
- No effect on post-weaning weights, achievement of sexual maturity, locomotor activity, auditory startle response, learning and memory, or reproductive performance in F1 litters (no HD offspring studied)
- No impact of F0 drug treatment on F2 parameters

Study no.: CD/2003/00331/00

Volume #, and page #: Module 4.2.3.5.3.1

Conducting laboratory and location: / /

Date of study initiation: 4 February 2003

GLP compliance: Letter included and signed

QA reports: yes (X) no ()

Drug, lot #, and % purity: GW572016F, Lot# 5720A4-02, purity not given

Methods

Doses: 20, 60 or 120 mg/kg/day

Species/strain: Rat/Wistar Han

Number/sex/group: 24 females/dose

Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 10 mL/kg volume

Satellite groups used for toxicokinetics: None

Study design: **F0 females** dosed from GD 6 – PND 20, with the day of mating noted as GD 0 and the day of parturition as PND 0. Females delivered naturally, then euthanized on PND 21.

F1 litters had 1 pup/sex/litter culled on PND 4, and litters were then culled to 3/sex/litter on PND 21. Each litter had 1 pup/sex assigned to:

Parameters and endpoints evaluated:

I – breeding for F2 litters

II – startle on PND 20 and activity on PND 61

III - startle on PND 60 and learning/memory
PND 62

II and III subsets were euthanized after
completion of tests and I subset males
euthanized after breeding and females
euthanized on PND 7

F2 litters euthanized on PND 7

F0 females: in-life observations, body weight,
food consumption, live pups counted, gross
necropsy, uterine former implantation sites, or
uterine contents plus corpora lutea and
implantations in any female that did not
deliver a litter by GD 25 or with total litter
loss

F1 rats: fetal external examinations, gender,
toxicokinetics, body weights, balano-preputial
separation, vaginal opening, startle reflex,
motor activity, swim T-maze, mating and
parturition, necropsy on unscheduled deaths

F2 rats: gender, weights, external exams and
dead pups given lung float test to determine if
stillborn then no further examination if no
external malformations seen

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Results

F₀ in-life:

Clinical observations noted that were likely due to GW572016 were limited to red discharge around the eyes, nose and/or mouth seen in the MD and HD rats.

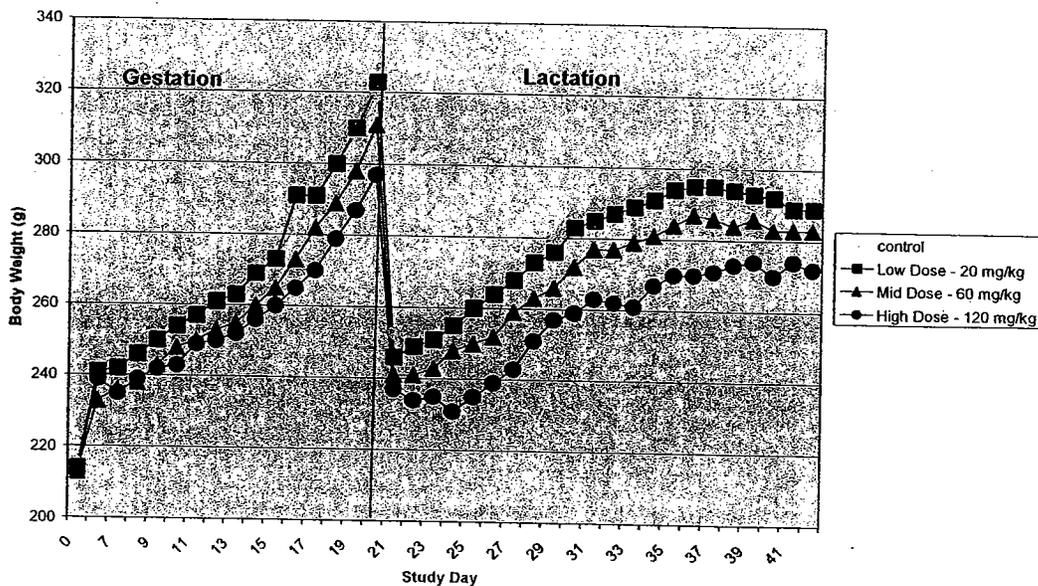
Seventeen HD rats had total litter loss between PND 0 – 5 and were euthanized. No rats died while on study otherwise. The table below shows the outcome of the breeding and shows that there was no difference in the pregnancy index among groups and that at the time of parturition, there was no effect of GW572016 on litter sizes or number of live pups. But soon after birth, the pups exposed *in utero* to the HD of GW572016 began dying and eventually there was a large increase in litter loss, with only 22% of the HD females with viable pups and 74% of the females with total litter loss by the end of the weaning period.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|---|-----------|----------------------|----------------------|------------------------|
| Females bred | 24 | 24 | 24 | 24 |
| Gravid females (%) | 23 (95.8) | 23 (95.8) | 22 (91.7) | 23 (95.8) |
| Females not gravid | 1 (4.2) | 1 (4.2) | 2 (8.3) | 1 (4.2) |
| Mean litter size | 11.1 | 11.9 | 10.7 | 11.1 |
| Mean live litter size* | 11.1 | 11.9 | 10.7 | 11.0 |
| Females with eventual total litter loss (%) | 0 (0) | 0 (0) | 0 (0) | 17 (73.9) |
| Females with viable pups at PND 21 (%) | 23 (100) | 23 (100) | 22 (100) | 5 (21.7) |

* - includes pups with positive lung flotation results, which indicates not a stillbirth

The graph below shows the body weights during gestation and lactation of the treated maternal F₀ rats. Significant decreases in body weight and body weight changes were seen in the HD rats during gestation. During lactation the HD rats had significantly lower body weights than the control rats, though this was likely due to the lower gestational body weights as body weight changes were not significantly different. In addition, these rats ate significantly less food, likely due to the decreased nutritional needs as their litters were dying to very large degrees. The MD rats had lower body weights than control during gestation, though not statistically significant, and the difference existed before dosing began. The significant change in the HD body weights is possibly an indicator a maternal toxicity, as litter sizes were comparable across groups. However the HD pups did weigh significantly less on PND 1 than control so this decrease in maternal body weights could be do to the smaller size of the HD neonates. Though PND 0 weights were not given and the pup survival data shows that dramatic effects are happening in these litters on the very first day of life as 75% of the pups died from PND 0 to PND 1. Without the PND 0 pup weights, it is inconclusive as to whether the decrease in maternal body weights was due to maternal toxicity or body weights of the pups.

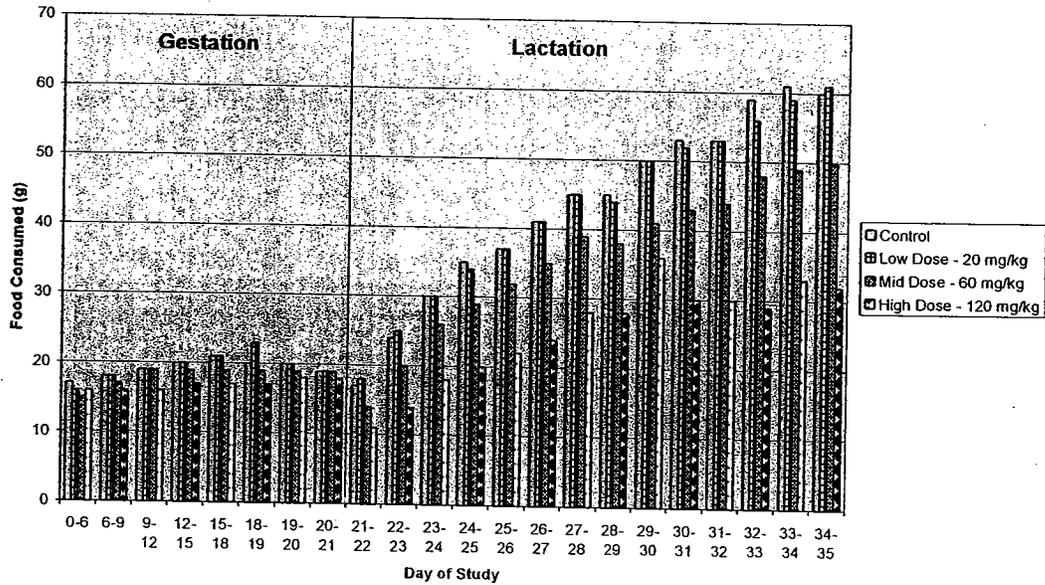
Maternal Body Weights During Gestation and Lactation



Food consumption was decreased in the HD group throughout gestation and in the MD group during several time points during gestation. During lactation, food consumption was significantly lower in the HD group throughout and in the MD group during the majority of the lactation period.

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Maternal Food Consumption During Gestation and Lactation



F₀ necropsy:

No drug-related gross necropsy findings were noted.

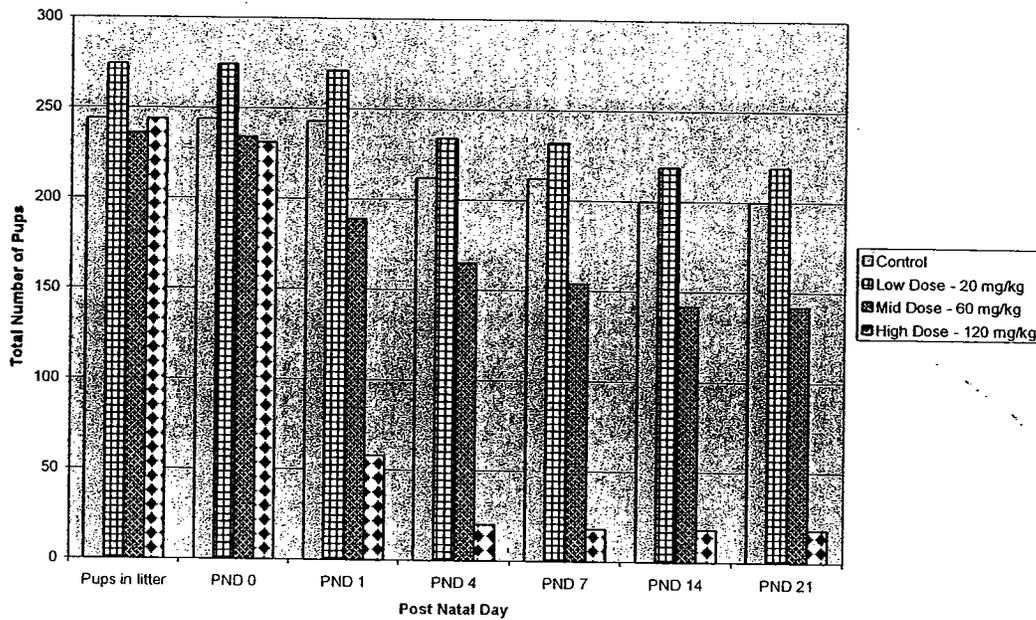
The parturition parameters are presented in the table below and show that no significant drug effect was seen.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
|------------------------------------|---------|----------------------|----------------------|------------------------|
| Gestation length (days) | 21.6 | 21.7 | 21.5 | 21.6 |
| Implantation sites | 11.9 | 12.6 | 11.4 | 11.7 |
| Number pups born | 11.1 | 11.9 | 10.7 | 11.1 |
| Unaccounted for implantation sites | 0.7 | 0.7 | 0.7 | 0.6 |
| Live litter size | 11.1 | 11.9 | 10.7 | 11.0 |
| Percent males/litter | 54.5 | 53.0 | 49.4 | 47.2 |

Postnatal survival of the F1 litters was significantly affected by GW572016 *in utero* treatment. The graph below shows the total number of viable pups at several time points during lactation. A significant effect of the MD and HD of GW572016 on the survival during the first four days after birth is seen. Postnatal survival was not different from control in the MD and HD group during the PND 7 to PND 14 time frame. There were

less viable pups at these points, but the deaths had occurred from PND 0 to PND 4.

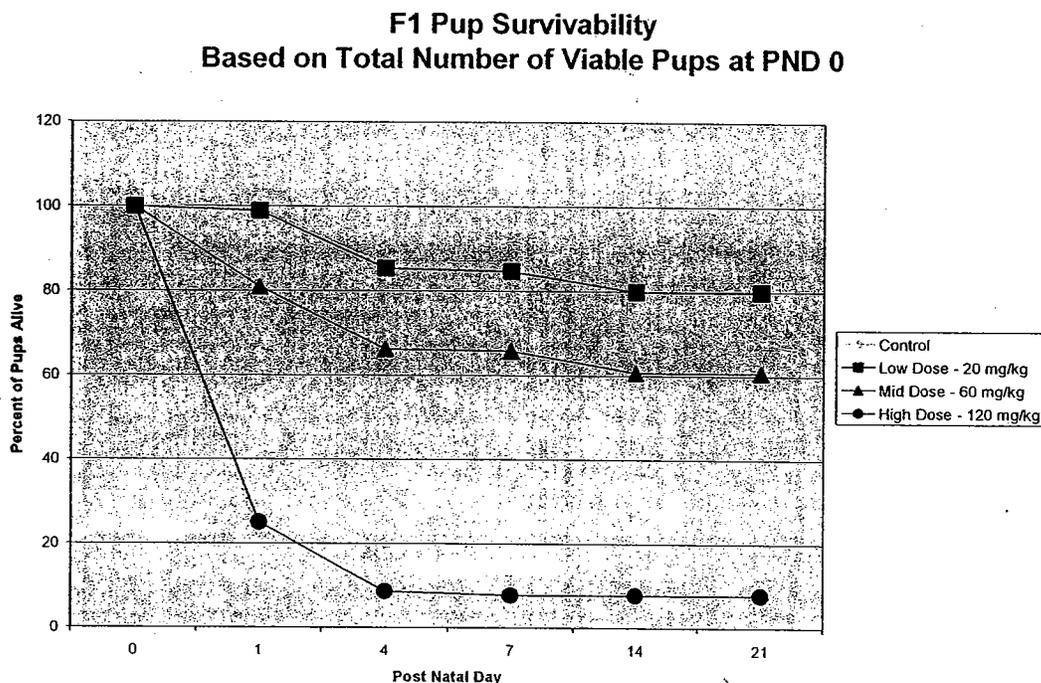
Total Number of Viable Pups in the F1 Litters



The table below shows that pup viability is severely affected by maternal GW572016 administration. Using as the denominator for the calculation the total number of viable pups in each dose group on the day of parturition, factoring out the number of pups that were born dead, the number of pups still alive at each given day prior to weaning is used as the numerator. Within one day of birth, 75% of the HD pups are dead and by Day 4 approximately 91% of the pups are dead. An effect on survival is also seen in the MD, though to a much lower degree.

| F1 Pup Survival Data | | | | |
|--|--------------------|------------------------------|------------------------------|--------------------------------|
| Pups Viable On Each Day – Based On Number Of Pups Delivered Viable | | | | |
| (Percent Of The Pups Born Alive That Were Still Alive At That Time Point) | | | | |
| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg | High Dose 120 mg/kg |
| PND 1 | 243/244 (99.6%) | 271/274 (98.9%) | 189/234 (80.9%) | 58/231 (25.1%) |
| PND 4 | 212/244 (86.9%) | 234/274 (85.4%) | 155/234 (66.2%) | 20/231 (8.7%) |
| PND 7 | 212/244 (86.9%) | 232/274 (84.7%) | 154/234 (65.8%) | 18/231 (7.8%) |
| PND 14 | 200/244 (82%) | 219/274 (79.9%) | 142/234 (60.7%) | 18/231 (7.8%) |
| PND 21 | 200/244 (82%) | 219/274 (79.9%) | 142/234 (60.7%) | 18/231 (7.8%) |

The data from the previous table is presented in graphical form below. Again, the effect of maternal GW572016 treatment on pup survivability is very apparent.



F₁ physical development:

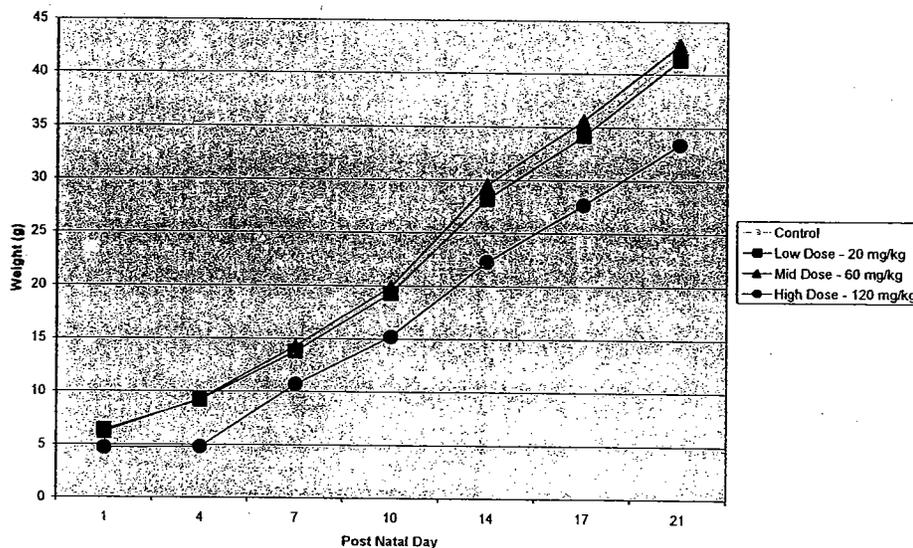
One pup/sex/litter were culled on PND 4 and examined for skeletal malformations. No effect of maternal GW572016 treatment was seen on skeletal development in the offspring.

Blood was taken from Control, LD and MD pups on PND 10, 2/sex/litter/timepoint, at 3, 8 and 22 hrs after dosing of the lactating F0 rat. While no GW572016 was detected in the control pups, it was present in the LD and MD pups. The presence of GW572016 in the F1 rats indicates that it is possible that GW572016 is being secreted in the milk and the pups are continuing to be exposed to some degree to GW572016. These levels could also be due to continued circulation of GW572016 from *in utero* exposure that is possibly not being cleared from the system of the pups as efficiently as it would in an adult rat.

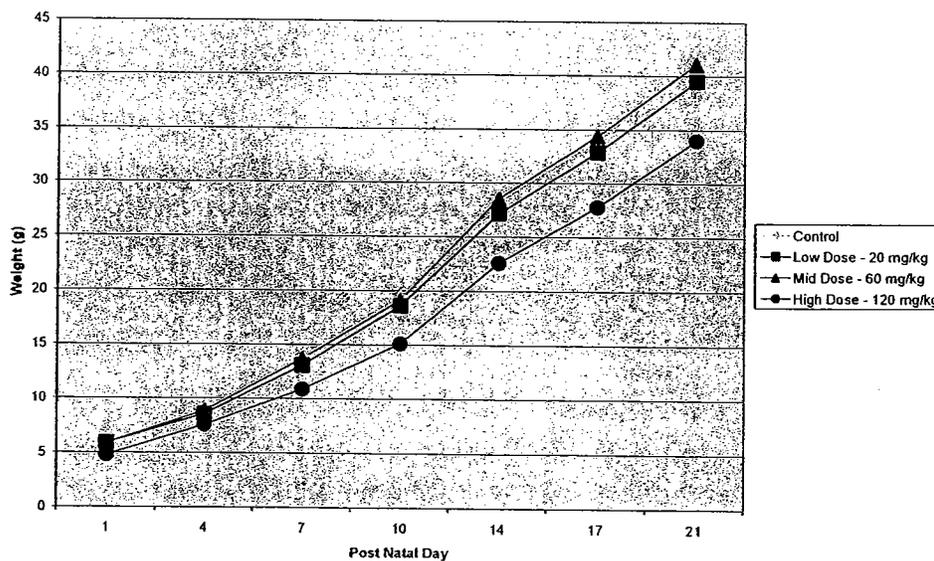
| Mean GW572016 Plasma Concentrations in F1 litters (ng/mL) | | |
|---|----------------|----------------|
| | LD – 20 mg/kg | MD – 60 mg/kg |
| PND 3 | Not Calculated | Not calculated |
| PND 8 | 19.5 | 46.1 |
| PND 22 | 10.7 | 36.3 |

Body weights of the F1 rats are presented below, with separate graphs for male and female rats. Weights of the HD pups were significantly lower throughout lactation than the control group. The post-weaning body weights are not presented, but no significant differences were seen in this time frame, though it should be noted that there were no HD pups during the post-weaning period due to the significant postnatal survivability problem with the HD litters

F1 Rat Pups Body Weights - Males



F1 Rat Pups Body Weights - Females



External and visceral exams of the pups that were found dead in the PND 0-4 timeframe, and able to be necropsied (not autolyzed or cannibalized) showed that MD and HD pups, for the most part, were not stillborn based on the lung flotation examination. These pups did not have milk in their stomachs. Therefore, even if offspring are being exposed to GW572016 through the milk, it is not the reason for the deaths of these pups. These data indicate that the pups were born alive yet did not nurse or obtain any nutrition from the dams. This could be due to the purported role of ErbB2 on mammary gland development. Published literature has reported that ErbB receptor signaling is required for epithelial cell growth and functional differentiation in immature mammary epithelial cells.

Sexual maturity developmental landmarks were not affected by the maternal GW572016 administration. The post natal days that the balano-preputial separation and acquisition of vaginal patency occurred did not differ among Control, LD and MD offspring. There were insufficient HD offspring for evaluation.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg |
|--------------------------------|---------|----------------------|----------------------|
| Balano-preputial separation | 44.9 | 44.2 | 44.5 |
| Acquisition of vaginal patency | 32.7 | 32.8 | 33.6 |

F₁ behavioral evaluation:

Auditory Startle Reflex

On PND 20 and PND 60, pups were examined for the effect of *in utero* GW572016 exposure on an auditory startle response reflex. No effect was seen on PND 20. On PND 60, the LD male pups had a lower maximum response amplitude (Vmax) than control pups, yet no effect was seen on the LD females or the MD pups. There is likely no toxicological relevance of this single group showing a significant difference from control. No HD F1 offspring were tested in this study.

Locomotor Activity

Locomotor activity was measured on PND 61 and results show that the MD females had a significantly lower total motor activity counts than did control pups, though ambulatory motor counts were not affected. No other groups showed a significant effect of treatment on any of the locomotor activity parameters. No HD F1 offspring were tested in this study.

Learning and Memory

The Biel maze (a T-maze swim test) was conducted on PND 62. The mean time to escape the maze and the mean number of errors committed in each of the 12 trials did not differ from Control in the LD or MD offspring. No HD F1 offspring were tested in this learning and memory study.

F₁ reproduction:

There were no F1 breeding animals for the HD group. No significant effect was seen on reproductive parameters in the LD and MD groups when compared to control. Mating and fertility indices are shown in the following table, as well as pre-coital interval, estrus cycle and gestation lengths.

| | Control | Low Dose 20 mg/kg | Mid Dose 60 mg/kg |
|--------------------------|---------|----------------------|----------------------|
| Male mating index | 100 | 100 | 100 |
| Female mating index | 100 | 100 | 100 |
| Fertility index | 95.2 | 100 | 95.5 |
| Mean pre-coital interval | 2.1 | 3.5 | 2.5 |
| Mean estrus cycle | 4.0 | 4.0 | 4.0 |
| Mean gestation length | 21.9 | 22.1 | 22.0 |

No treatment-related effects on external or internal macroscopic examinations of the F1 rats that were used for the behavioral and reproductive segments of the study were seen.

F₂ findings:

No significant effects of the F0 GW572016 treatment was seen on F2 litter sizes, mean number of live pups, percentage of litters that were male, postnatal survival, mean pup body weights or body weight gains from PND 1 to PND 7, or on external and visceral examinations.

Study title: GW572016F: Oral cross-fostering study in rats.

Key study findings:

- Study was conducted to look at the potential for an adverse effect of GW572016 treatment on mammary glands of the dams which could lead to ↓ nursing of the litters and ↓ pup viability
- No effect of GW572016 on mammary gland histology
- Significant effect of GW572016 treatment during gestation on postnatal pup viability, regardless of whether the pups are nursing from a dam that received GW572016 or not
- Pup viability effects seen in this and other studies are not due to mammary gland effects
- Postnatal growth retardation is seen in pups exposed only *in utero* to GW572016 an also in pups exposed only through milk to GW572016.

Study no.:

CD/2004/00609/00

Volume #, and page #:

Module 4.2.3.5.3.2

Conducting laboratory and location:

/ /

Date of study initiation: 7 January 2004
GLP compliance: Non-GLP
QA reports: yes () no (X)
Drug, lot #, and % purity: GW572016F, Lot# R5361/144/1, purity not given

Methods

Doses: Control1 0
Control2 0
GW572016 120 mg/kg/day

Species/strain: Rat/Wistar Han

Number/sex/group: 24 females/dose

Route, formulation, volume, infusion rate: PO, in 0.5% hydroxypropyl methylcellulose + 0.1% Tween 80, 10 mL/kg volume

Satellite groups used for toxicokinetics: None

Study design: **F0 females** dosed from GD 6 – PND 7, with the day of mating noted as GD 0 and the day of parturition as PND 0. There were two Control groups and one GW572016 group with 15 dams/group. Females delivered naturally, then within 2 hrs each litter from one Control group (Control2) were cross-fostered to a GW572016-treated dam and the GW572016 dam's litters were cross-fostered to the Control dams. The other Control group (Control1) weaned the litters they delivered. There were an additional 10 dams given vehicle or GW572016 (5 dams/group) and pups and dams were euthanized after parturition and used for examination of the histology of the mammary glands.

F1 litters were euthanized on PND 8

Parameters and endpoints evaluated: **F0 females:** in-life observations, body weight, food consumption, gestation length, necropsy findings
F1 rats: litter size and survival, body weights, clinical observations, external and visceral examination

F₀ in-life:

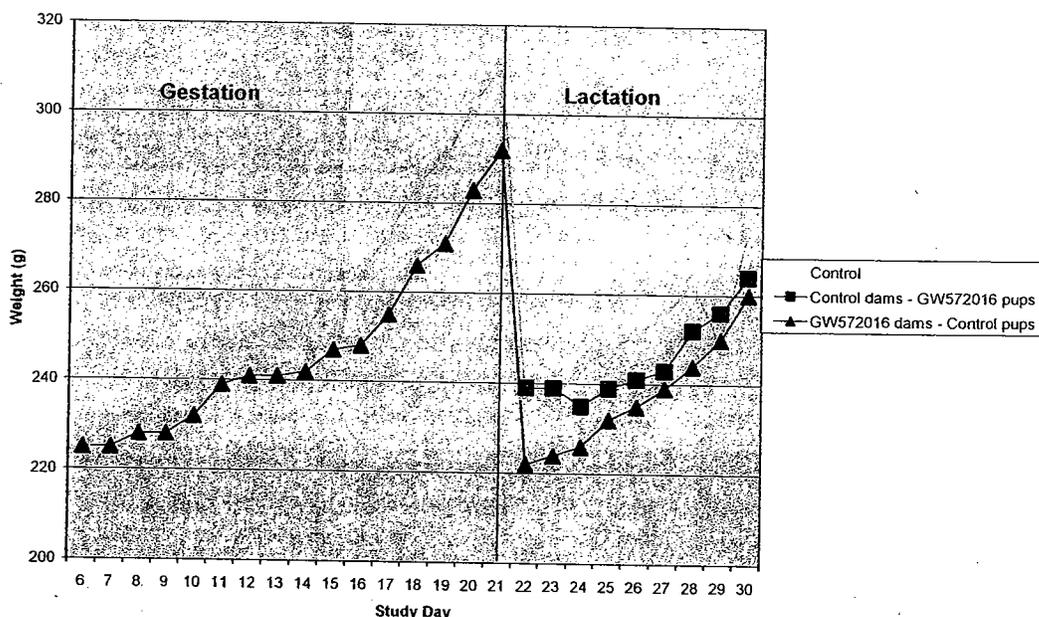
Between PND 0 and 5, total litter loss was seen in 2/15 Control1 litters where the dams received vehicle and nursed their own litters, 10/15 Control2 litters where the dams received vehicle but then nursed litters that had GW572016 exposure in utero and 1/15 GW572016 litters where the dams received GW572016 while pregnant but then nursed pups that had no drug exposure *in utero*. There was also one Control1 dam found dead

on PND1 and one Control1 dam that failed to deliver and was euthanized on GD25. In summary, the offspring in the Control2 group, with a large number of total litter losses, were cross-fostered to dams that were being treated with GW572016, so this litter loss that was seen in a control group is considered to be GW572016-related.

No clinical observations attributable to GW572016 treatment was seen in the F0 female rats.

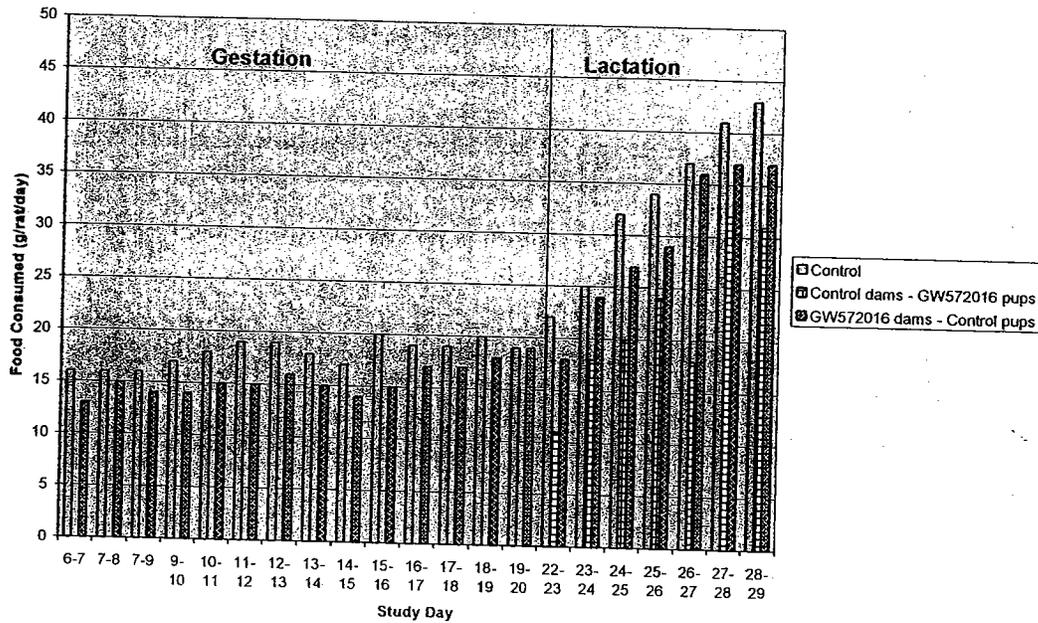
Maternal body weights are presented in the graph below. During gestation, both control groups are presented as one line (Control1 with control exposed litters and Control2 with cross-fostered GW572016 exposed litters). During the lactation period, these groups are presented separately. Starting on GD 13 and continuing through the remainder of gestation and through lactation, the F0 rats treated with GW572016 have significantly lower body weights when compared to the control rats (compared to the control rats with no cross-fostering during lactation). The decreased body weights in the GW572016 group during lactation is attributable to the decreased weight gain during gestation.

F0 Maternal Body Weights During Gestation and Lactation



Food consumption was reduced in the GW572016 treated dams during gestation. During lactation, mean food consumption was lower in the GW572016 treated dams as well as in the control dams fostering pups from the GW572016 treated dams. The decreased food consumed in the GW572016 treated dams is most likely a direct drug effect. The females that were treated with the control vehicle but fostering pups from GW572016 litters, likely ate less food than the controls that were not cross-fostering because their food needs were less due to the smaller litters due to the effect of *in utero* GW572016 exposure on postnatal viability.

F0 Food Consumption During Gestation and Lactation



No drug effect on the length of gestation was seen.

F₀ necropsy:

No drug effect as seen on the histology of the mammary glands. These were examined given the literature reports of the role of ErbB in mammary gland development and the potential for the maternal GW572016 administration to impact the mammary glands leading to lack of nursing in the litters of GW572016 treated dams and a decrease in pup viability.

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F₁ physical development/litter observations:

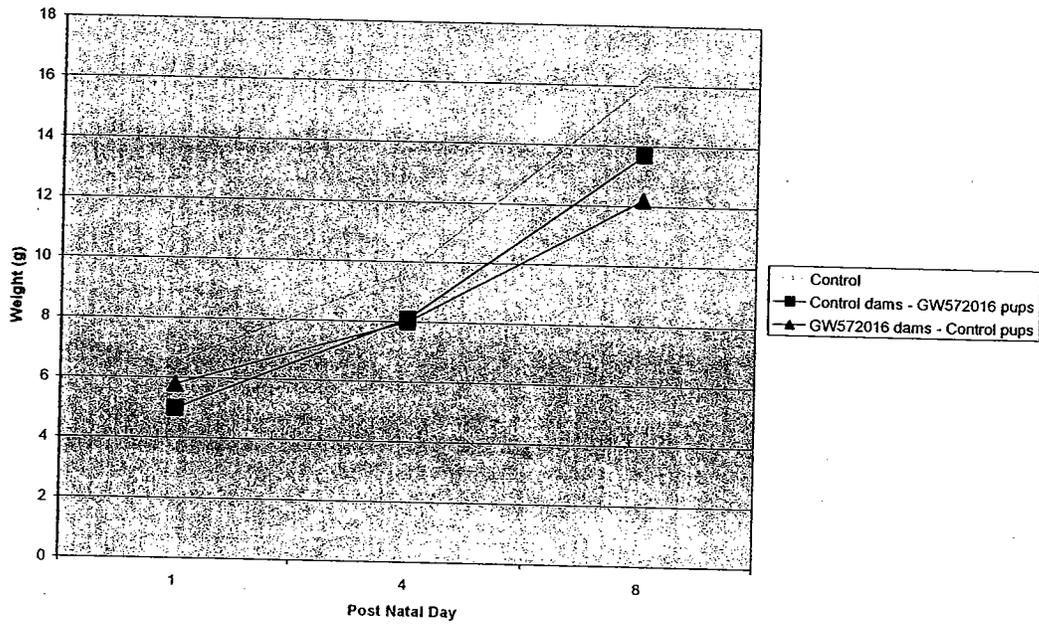
There was no effect on the treatment group on the number of pups born, the mean percentage of males per litter at birth or in the live litter sizes.

There was an impact of *in utero* GW572016 exposure on pup viability. Just as pups that were exposed *in utero* and nursed by GW572016-treated dams had 91.3 % of the pups dead by PND 4, in this study pups that were exposed *in utero* then nursed from dams that had no GW572016 exposure had 81.7% of the pups dead by PND 4 and 82.4% of them dead by PND 8.

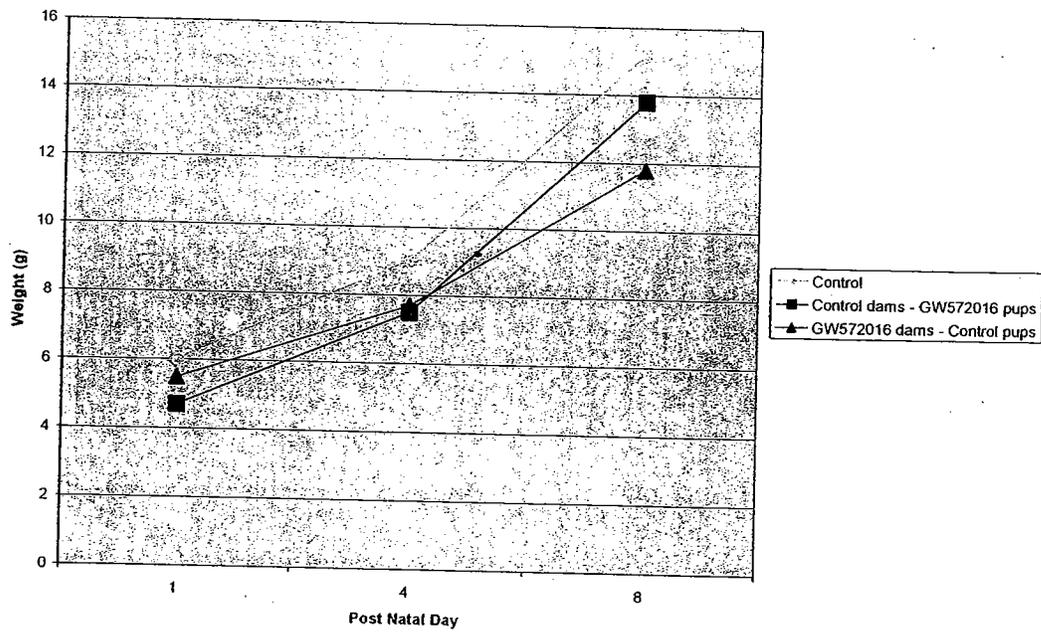
| | Control Dams Control Pups Control Dams nursing their own Control Litters | Control Dams GW572016 Pups Control Dams nursing pups that were exposed <i>in utero</i> to GW572016 | GW572016 Dams Control Pups GW572016 treated Dams nursing pups that had no GW572016 <i>in utero</i> exposure |
|---|--|--|---|
| Percent of litter dead at PND 8 (based on number alive at PND 0) | 14.3 % | 82.4 % | 13.1 % |
| Mean number born | 8.6 | 8.9 | 9.7 |
| Mean number alive PND 0 | 8.5 | 8.7 | 9.7 |
| Number pups alive PND 4/Number alive PND 0 (%) | 107/119 (99.2%) | 23/131 (18.3%) | 128/145 (88.3%) |

The weights of the F₁ pups are presented in the two graphs below. Significant differences were seen in the Control/GW572016 group and the GW572016/Control group when compared to the Control/Control group. Pups that were exposed to GW572016 *in utero* and pups that although not exposed *in utero* were cross-fostered to dams that were still being administered GW572016 all weighed significantly less than pups that had no chance of any exposure, during gestation or lactation, to GW572016. The decrease in body weights in the pups born to control dams but fostered by GW572016 dams could possibly be due to GW572016 exposure from the milk but it seems unlikely. The difference occurs on PND 1 but then the Control/Control and Control/GW572016 pups gain weight at an equal pace throughout the next 7 days, unlike the GW572016/Control pups. Without fetal weights for PND 0 it is difficult to tell if that one day of nursing from a GW572016-treated dam is the reason for the decreased body weight of the Control/GW572016 pups on PND 1.

F1 Male Body Weights



F0 Female Body Weights



External and visceral examinations of the pups that were found dead showed a significant increase in the number of pups in the Control dams/GW572016 pups group that did not have milk in their stomachs. Given that these pups were immediately cross-fostered to dams not treated with GW572016, this can not be due to any impact of an ErbB2 inhibitor on mammary gland development. No other significant external or visceral effects were seen.

F₁ behavioral evaluation:

Not conducted

F₁ reproduction:

Not conducted

F₂ findings:

None

2.6.6.7 Local tolerance

RD2000/00530/00: GW572016F: Acute dermal irritation study in the New Zealand White rabbit

Three male rabbits were used to examine the potential of GW572016 to cause dermal irritation. A 2.5 cm square gauze patch moistened with distilled water and 0.5 g of GW572016 was applied to the intact skin of the rabbits. After a 4 hr exposure period the gauze patch was removed, the site cleaned of residual drug material and scored according to the Draize classification. The sites were observed for 72 hrs after the patch was removed. The Draize classification is as follows: 0=non-irritant, >0-2= mild-irritant, >2-5= moderate irritant and >5-8= sever irritant. Under the conditions of this study, GW572016 yielded a Draize classification of 0.3, due to a very slight erythema and edema noted in one of the rabbits, which had resolved by the 72 hr endpoint.

RD2000/00531/00: GW572016F: Acute eye irritation study in the New Zealand White rabbit

Four male rabbits were used to examine the potential of GW572016 to cause eye irritation. One rabbit received 10 mg of GW572016 and the other three rabbits received 26 mg applied to the everted lower lid of the right eye, with the left eye left untreated. The upper and lower lids were held together for 1 minute after article administration and the eye remained unflushed. The eyes were observed at 1, 3, 6, 24, 48, and 72 hrs post-treatment. Any irritation was graded and scored according to the Draize classification. Additionally, examinations were conducted at 24 hrs to determine any corneal injury. Overall Draize scores of 6.0 for 10 mg and 10.0 for 26 mg were determined. These scores are classified as a Grade I, which is considered negligible risk of eye damage, according to the Draize technique. All rabbits were negative for any corneal injury. Under the conditions of this study, GW572016 poses a negligible risk to the eye.

RD2000/00532/00: GW572016F: Skin sensitization (Magnusson-Kligman) study in the guinea pig

Albino guinea pigs were used to evaluate the potential for skin sensitization by GW572016 using the Magnusson-Kligman maximization method. Guinea pigs were given an intradermal injection of 5.0% w/v of the test article in mineral oil or Freund's Complete Adjuvant (FCA)/sterile water on Day 1. On Day 8, 75% w/v of the test article in mineral oil was applied topically over the injection site for 48 hrs. On Day 22, 2 weeks after the topical induction phase, the animals received a challenge dose of GW572016 with 75% w/v in mineral oil applied to the skin for 24 hrs. On Day 24 the challenge sites of the skin were examined for any dermal reactions and then examined again on Day 25. Moderate irritation was noted at the intradermal injection sites and mild to moderate irritation noted at the topical induction sites. Mild reactions were noted at both sites in a control group tested with the vehicles only. None of the guinea pigs exhibited any reaction to the challenge application of GW572016. Under the conditions of this study, GW572016 was not a dermal sensitizer.

Local Tolerance with GW572016 impurities**RD2003/00061/00: — Rabbit enucleated eye test**

The ocular irritancy potential of — a genotoxic material used in the synthesis of lapatinib that is present beyond acceptable limits in the final drug product, was tested in the rabbit enucleated eye test. Rabbits were euthanized and the eye dissected out and placed within the chamber of a superfusion apparatus. Three eyes were treated with the test material, and two other eyes untreated and used as controls. Observations were conducted at 60, 120, 180, and 240 minutes after treatment. No effect of — exposure was seen on corneal opacity, fluorescein uptake by the corneal epithelium or on corneal thickness and the overall condition of the cornea. The results of this *in vitro* study indicates that — has little potential to cause ocular irritancy *in vivo*.

RD2005/01536/00: — Acute dermal irritation in the rabbit

The potential for dermal irritation of — a genotoxic material used in the synthesis of lapatinib that is present beyond acceptable limits in the final drug product was tested in the rabbit. A single 4-hr semi-occluded application of 0.5 g of the test material was applied using a gauze patch on the shaved skin of three rabbits. The skin was monitored for signs of irritation at 24, 48 and 72 hrs after the removal of the gauze patch. There was no evidence of irritation as the three rabbits had a primary irritation index of 0, which indicates an irritancy classification of "non-irritant". One rabbit with three patches that were analyzed after exposure times of 4 hrs, 1 hr or 30 minutes, also had irritation scores of zero. This study indicates that — is not likely to be a dermal irritant or corrosive agent.

RD2003/00063/00: — Rabbit enucleated eye test

The potential for ocular irritation of —, a genotoxic material used in the synthesis of lapatinib that is present beyond acceptable limits in the final drug product, was tested in the rabbit. Rabbits were euthanized and the eye dissected out and placed within the chamber of a superfusion apparatus. Three eyes were treated with the test material, and two other eyes untreated and used as controls. Observations were conducted at 60, 120, 180, and 240 minutes after treatment. No effect of — exposure was seen on corneal opacity, fluorescein uptake by the corneal epithelium or on corneal thickness and the overall condition of the cornea. The results of this *in vitro* study indicate that — has little potential to cause ocular irritancy *in vivo*.

RD2005/01537/00: — Acute eye irritation in the rabbit

The potential for ocular irritation of — a genotoxic material used in the synthesis of lapatinib that is present beyond acceptable limits in the final drug product, was tested in the rabbit. An application of 0.1 ml (87 mg) of the test compound was placed into the conjunctival sac of the right eye and the eye lids were held together gently to avoid loss of test material. The left eye was not treated. Pain reaction was assessed immediately after the test article was administered. Ocular damage/irritation was assessed at approximately 1, 24, 48 and 72 hrs after treatment by assigning a numerical evaluation based on the Draize technique. The initial pain reaction score showed a reaction indicative of a "slight initial pain". Minimal to moderate irritation was noted in the conjunctivae at the 1 and 24 hr time points. The results of the study show — would be classified as a "minimal irritant".

RD2005/01538/00: — skin sensitization in the guinea pig – Magnusson and Kligman maximization method

The potential for contact sensitization of — a genotoxic material used in the synthesis of lapatinib that is present beyond acceptable limits in the final drug product, was tested in the guinea pig using the method of Magnusson and Kligman. The guinea pigs received an injection of a 5% w/w formulation of — in the intradermal induction phase on Day 1, then a 48-hr topical exposure of a 50% w/w formulation of — at the same location on Day 7 for the topical induction phase and then on Day 21 a 48-hr topical application of a 50% w/w formulation of — for the challenge phase. After the intradermal induction phase the sites were monitored at 24 and 48 hrs after the injection for 1 and 24 hrs after the removal of the patch in the topical induction phase and for 24 and 48 hrs after removal of the patch in the challenge phase. The results of the induction phase showed that — at the concentrations tested caused erythema and swelling or edema to varying degrees, though the concentrations were chosen based on inducing mild to moderate irritation in a concentration selection study. The results of the challenge phase of the study showed that — did not cause any challenge reaction and was classified as a "non-sensitizer" under the conditions of this test.

2.6.6.8 Special toxicology studies

Study title: GW572016F: Effect on anti-KLH antibody response in a 28-day oral dose immunotoxicity study in the rat

Key study findings:

| | |
|--|--|
| Study no.: | CD2004/00055/00 |
| Volume #, and page #: | Module 4.2.3.7.2.1 |
| Conducting laboratory and location: | GlaxoSmithKline Safety Assessment – Upper Merion 709 Swedeland Road King of Prussia, PA 19406 |
| Date of study initiation: | 24 October 2003 |
| GLP compliance: | Letter included and signed |
| QA reports: | yes (X) no () |
| Drug, lot #, and % purity: | GW572016F, Lot# R5361/144/1, purity not listed |
| Formulation/vehicle: | 0.5% hydroxymethylcellulose + 1% Tween 80 |

Methods

Doses:

Male rats- 0, 60 and 180 mg/kg/day

Female rats – 0, 20 and 160 mg/kg/day

Study design:

- 10 Wistar-Han rats/sex/dose were used in the study
- Drug administered PO daily for 28 days
- Immune function was assessed by measuring the primary antibody response to the T-cell dependent antigen, keyhole limpet hemocyanin (KLH), following a single immunization by IV injection. Immunization with KLH occurred on Day 14 with 600 µg/kg administered by injection in the tail vein.
- Body weights monitored daily
- Blood obtained on Day 19 and Day 29 for measurement of serum anti-KLH IgM and IgG antibody concentrations by ELISA

Results:

- Modest decrease in body weight gain from Day 1 to Day 28 in male and female HD rats when compared to control, but no significant difference in body weights
- Immunization with KLH induced comparable anti-KLH IgM and IgG antibody response in the drug-treated rats as in the control rats
- GW572016 did yield a geometric mean concentration (GMC) of anti-KLH IgM antibodies that was 20-30% lower than what was seen in the controls but this was not statistically significant and a similar result was not seen in the more mature IgG antibody response

- An immunosuppressive drug, cyclosporin A, in a study by the sponsor that is in preparation, showed a statistically significant decrease of $\geq 60\%$ in both anti-KLH IgM and IgG
- Given the results and what has been seen with known immunosuppressive drugs, it is not believed that GW572016 would adversely affect human immune function

2.6.6.9 Discussion and Conclusions

A full battery of toxicology studies has been conducted with lapatinib in nonclinical models. The primary toxicities of lapatinib in the laboratory are the skin, GI tract and accessory digestive organs, mammary glands, prostate and the liver. The general toxicology program has adequately addressed the safety of lapatinib with appropriate animal models and dosing ranges and regimens.

The majority of the toxicities seen with lapatinib are most likely an extension of the pharmacological action of the drug. Skin lesions and reddening, seen in the rat and dog, are known actions of drugs of this class. Other EGFR tyrosine kinase inhibitors have this same effect and skin rashes are a primary toxicity seen in man. Although emesis and diarrhea were not widely seen in the toxicology studies, and loose stools were only noted in the rabbit, there were still significant histopathological changes on the GI, including degeneration and inflammation. These changes are likely due to the inhibition of the EGFR located within the gastric mucosa. Again, this toxicity is one also seen clinically, as diarrhea has been the DLT in clinical trials.

Two toxicities that have become of interest with drugs that act to inhibit tyrosine kinases are cardiac and ocular effects. In the studies with lapatinib, there was little evidence of these two toxicities being problematic. The only noted eye toxicities were muscular, with damage to the eye skeletal muscle seen; no corneal or retinal damage was found. ECGs were not adversely affected by lapatinib treatment. Histopathological changes in the hearts of rats and dogs were noted, but they were of a very low frequency. These included focal fibrosis, infiltration and myocyte degeneration of the heart in rats and one dog with hemorrhage noted in the heart. The rat finding was not replicated in a longer-term study at the same dose. In general, these results are not indicators of a strong possibility of potential cardiac toxicities from Tykerb in the clinic, though vigilance in monitoring the potential is warranted until more clinical experience is gained.

Lapatinib was not mutagenic or clastogenic in the battery of genotoxicity studies. It does have a genotoxic impurity that is present at levels that exceed recommended limits. As the Sponsor is currently conducting 2-yr carcinogenicity studies in both the rat and mouse, more information regarding the carcinogenic potential of lapatinib and this impurity are forthcoming, provided the carcinogenicity animals are documented to have been exposed to adequate levels of the impurity.

In the integrated summary presented by the sponsor, testicular and epididymal sperm counts and sperm motility are listed under "data collected" for the male fertility study in the rat. The full study report did not include any information on these parameters, so technically gonadal function was not evaluated in this study. However there were no

effects on the mating and fertility indexes or on the C-sectioning results in the un-treated females. There were also no clear signs of toxicity on the testes in any of the general toxicology studies with the rat or dog, with minimal histological changes in other accessory sex organs in both species. Given all the information that is known, it is highly unlikely that there would have been any effects of lapatinib treatment on these gonadal function parameters.

The pivotal embryo-fetal development study in the rats showed maternal toxicity that was inconsistent with, or not what would have been expected from, the dose range-finding study used to set the doses. In the pilot study, decreases in body weights were clearly evident through most of gestation. Additionally, body weight gain during gestation was decreased upwards of 30-50% compared to control at 720 mg/m² and a dose of 1080 mg/m² was clearly too toxic as all dams were euthanized moribund. The choice of 720 mg/m² was perfectly acceptable as the high dose for the pivotal trial and given the results at the 1080 mg/m² dose it is unlikely a dose higher than 720 mg/m² would have supported a pregnancy. In the pivotal studies, the body weights were affected, but the HD dams weighed only 5% less than the control on GD 21, unlike in the pilot study where the HD weighed 10% less than control. But in the pivotal study, when body weight gains are compared for the GD 7 – 18 timeframe, the HD rats are gaining 31% less weight than the control rats. The sponsor did not present the corrected maternal body weights at GD 21, the weight of the mother minus the gravid uterus. This information is needed to determine if body weight effects in the dam are indicative of maternal toxicity or of embryo-fetal lethality. As no significant effects were seen on fetal body weights or number of live fetuses/litter, it would appear that the body weight gain effects are related to maternal toxicity.

The most striking reproductive toxicity effect was seen in the pre- and post-natal development study. The HD of 720 mg/m², used in several other reproductive toxicology studies produced a striking decrease in neonatal viability. While there was no difference in the number of viable fetuses born, 91% of the HD offspring died within the first four days of life. The sponsor further researched the potential that this effect was due to mammary gland development, as erbB-2 is known to be involved, and because the pups that died did not have milk in their stomachs. A cross-fostering study did not confirm this, as 84% of pups exposed *in utero* to lapatinib still died, despite being nursed by dams that had never been exposed to lapatinib. This lethality is unusual and further research to determine exactly what may be occurring would be highly informative. It would be interesting to see what the critical gestational days are for lapatinib to yield this effect. This may better help physicians to counsel their patients regarding pregnancy and Tykerb administration. More thorough examinations of the dead offspring may also shed some light on the actual cause of the neonatal lethality.

2.6.6.10 Tables and Figures
See text of review for pertinent tables and figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

| <i>Acute Toxicity Studies</i> | | | | | |
|-------------------------------------|--------------------------|------------|-----------|------------------------|--|
| Species | Route | N/sex/dose | mg/kg | mg/m ² | Significant findings |
| Mouse | Oral | 9 | 2000 | 6000 | 6000 mg/m ² : no toxicity |
| Rat | Oral | 9 | 2000 | 12000 | 1200 mg/m ² : slight ↓ in body weight and body weight gain |
| Mouse | IV | 6 | 46 | 138 | 138 mg/m ² : no toxicity |
| | | 3 | 27 | 81 | 81 mg/m ² : no toxicity |
| Rat | IV | 6 | 21.2 | 127.2 | 127.2 mg/m ² : no toxicity |
| | | 3 | 8.9 | 53.4 | 53.4 mg/m ² : GI mucosal inflammation |
| <i>Repeat Dose Toxicity Studies</i> | | | | | |
| Species | Route Duration | N/sex/dose | mg/kg/day | mg/m ² /day | Significant findings |
| Mouse | Oral Daily x 14 | 10 | 1000 | 3000 | 3000 mg/m ² /day: all euthanized moribund |
| | | | 300 | 900 | 900 mg/m ² /day: ↓ RBCs, Hct, Hb (♂) and ↑ reticulocytes in ♀ |
| | | | 100 | 300 | 300 mg/m ² /day: no toxicity |
| Mouse | Oral Daily 13 Week | 28 | 200 | 1200 | 120 mg/m ² /day: liver hypertrophy, cecum and colon hyperplasia, preputial gland inflammation and bone marrow hypercellularity. spleen hematopoiesis |
| | | | 100 | 600 | 600 mg/m ² /day: 1 ♂ died, : liver hypertrophy, cecum hyperplasia, preputial gland inflammation, spleen hematopoiesis |
| | | | 50 | 300 | 300 mg/m ² /day: minimal spleen hematopoiesis |
| Rat | Oral Daily x 7 | 3 | 240 | 1440 | 1440 mg/m ² /day: ↑ adrenal weights, ↓ prostate weights, prostate infiltrate and atrophy, kidney regeneration and dilatation, liver infiltrate, esophagus skeletal muscle degeneration, lung infiltrate, lymph node hemorrhage |
| | | | 120 | 720 | 720 mg/m ² /day: ↑ adrenal weights, ↓ prostate weights, prostate infiltrate |
| | | | 60 | 360 | 360 mg/m ² /day: ↑ adrenal weights, ↓ prostate weights, prostate infiltrate and atrophy |
| Rat | Oral Daily x 14 | 10 | 1000 | 6000 | 6000 mg/m ² /day: mortality (7% ♂ and 46% ♀), dehydration, ↓ activity, red discoloration, loose feces, ↓ BW and food, ↑ WBC parameters, ↑ AST, ALT and bile acids, ↓ urine volume (♂), ↓ thymus and prostate size and weight, erosion ulceration of GI, liver necrotizing inflammation, salivary gland atrophy, thymus lymphoid depletion, tongue and eye muscle degeneration, lung histiocytosis, mammary gland degeneration (♂) |
| | | | 240 | 1440 | 1440 mg/m ² /day: dehydration, ↓ activity, red discoloration, loose feces ♀ only except for loose feces, ↓ BW and food (♀), ↑ WBC parameters, ↑ |
| | | | 60 | 360 | |

| | | | | | |
|-----|-----------------------------|-----------|--|-------------------------------------|---|
| | | | | | AST, ALT and bile acids, ↓ prostate weight, erosion ulceration of GI, (♀), liver necrotizing inflammation (♀), salivary gland atrophy, lung histocytosis (♀), 360 mg/m ² /day: ↑ adrenal weights, ↓ prostate weights, prostate infiltrate and atrophy, ↑ WBC parameters (♀) |
| Rat | Oral Daily x 13 Weeks | 12 | 180 60 20 | 1080 360 120 | 1080 mg/m ² /day: mortality in ♀ though may be gavage error, salivation, clipped incisors dehydration, scabs, ↓ BW (♀), ↑ WBC parameters, ↑ AST, ALT and bile acids, ↑ liver, spleen and adrenals (1 ♀ rat), scabs (♀), ↑ spleen, lung and adrenal weights (♀), ↓ uterus weight, skin histopathology, GI mucosal degeneration, liver hypertrophy, hyperplasia and infiltrate, salivary gland atrophy, lymph node hyperplasia, heart fibrosis and myocyte degeneration (♂), mammary gland degeneration (♀), lung histocytosis, uterine horn atrophy 360 mg/m ² /day: salivation, ↑ WBC parameters, (♀), ↑ AST, ALT and bile acids, skin histopathology 120 mg/m ² /day: salivation, ↑ bile acids, skin histopathology |
| Rat | Oral Daily x 26 Weeks | 20 | 180 (120) 60 20 (10) (♀ dose) | 1080 (720) 360 120 (60) | 1080 (720) mg/m ² /day: 1 male death (other deaths likely due to blood draws), ↑ WBCs, ↑ ALT, bile acids, cholesterol, enlarged lymph nodes (♀), scabs (♀), ↑ adrenal, liver and kidney weights (♀), skin histopathology (♀), lymphoid hyperplasia in lymph node (♀) 360 mg/m ² /day: ↑ WBCs (♀), ↑ ALT and bile acids and cholesterol (♀), enlarged lymph nodes (♀), scabs (♀), skin histopathology (♀), lymphoid hyperplasia in lymph node (♀) 120 (60) mg/m ² /day: no toxicity |
| Dog | Oral Daily x 7 | 3 | 120 60 30 | 2400 1200 600 | 2400 mg/m ² /day: scab, loose feces, enlarged spleen 1200 mg/m ² /day: loose feces, 600 mg/m ² /day: loose feces |
| Dog | Oral Daily x 14 | 5 HD 3 | 360 60 10 | 7200 1200 200 | 7200 mg/m ² /day: 40% mortality (♀), loose feces, vomiting, ↓ activity, dehydration, salivation, ↓ BW and food, ↑ WBCs and RBCs ↑ bile acids, bilirubin, ALT (♀) and alkaline phosphatase, ↓ adrenal weight, ↓ spleen, thymus, prostate, uterus, ovary and lung weights, GI mucosal degeneration and dilation, lymphoid depletion in spleen, lymph nodes and thymus, muscle atrophy (♂), zymogen depletion in pancreas, inflammation and ulceration of tongue and gingival, liver glycogen depletion 1200 mg/m ² /day: loose feces, ↑ alkaline phosphatase (♀), bile acids and ALT, ↓ spleen, thymus, ovary, liver, uterus and prostate weights, 200 mg/m ² /day: loose feces |
| Dog | Oral daily x 13 Weeks | 4 | 160 40 10 | 3200 800 200 | 3200 mg/m ² /day: 33% mortality (♂), ↓ activity, dehydration, loose feces, scabs, vomiting, ↓ BW and food, ↑ WBC parameters, ↑ alkaline phosphatase, bilirubin bile acids, ALT, cholesterol (♂), ↓ albumin, ↑ urobilinogen, distended gall bladder, ↑ adrenal, |

| | | | | | |
|-----|-----------------------------|---|-----------------|--------------------|--|
| | | | | | lung, pituitary and liver weight (♂), GI mucosal degeneration and Peyer's patches lymphoid depletion, liver glycogen depletion (♀), inflammation and ulceration of tongue (♀), salivary gland atrophy (♀), lymphoid depletion of lymph nodes, tonsil, spleen (♂) and thymus, cytoplasmic alteration of adrenal cortex, skin histopathology <u>800 mg/m²/day</u> : ↑ monocytes (♀) <u>200 mg/m²/day</u> : |
| Dog | Oral Daily x 39 Weeks | 4 | 100 40 10 | 2000 800 200 | <u>2000 mg/m²/day</u> : 50% ♂ mortality, severe BW loss, ↓ food consumption, dehydration, skin lesions, ↑ platelets and WBCs (♀) and ↓ RBC parameters, ↑ bilirubin, ALT, ALP, bile acids, globulin (♀), ↓ albumin, ↑ urobilinogen, ↑ spleen and lymph node size (♀), skin ulceration (♂), ↑ liver organ weight and relative brain and pituitary weights (♂), lymphoid depletion in GI and lymph node, bile duct hyperplasia/dilatation/inflammation (♂), cholelithiasis (♀), liver inflammation and degeneration/necrosis, skin histopathology <u>800 mg/m²/day</u> : ↑ ALT, ↑ lymph node size, <u>200 mg/m²/day</u> : ↑ heart weight |

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The non-clinical program of lapatinib identified the target areas of toxicity to be the skin, GI tract and accessory digestive organs, mammary glands, prostate and the liver. It was neither genotoxic or teratogenic. There is a genotoxic impurity in the drug product that exceeds recommended limits. Though not teratogenic, there is a significant amount of neonatal loss within the first week of life when rats are exposed *in utero* to lapatinib.

Unresolved toxicology issues (if any): None

Recommendations: Recommend that lapatinib (Tykerb) is approvable, with the preclinical studies adequately addressing the non-clinical safety requirements.

Suggested labeling:
Presented in a separate labeling review

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

None

**This is a representation of an electronic record that was signed electronically and
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/s/

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