Results:

- Clinical signs:
- Mortality: 0.2 mg/kg- 1 death on Gestation Day 19
- Body weight:

Mean Maternal Body Weight Changes (g/dam/day)

Group	Daily Dose (mg/kg)	n[a]	Days of Pregnancy 0-6	6-10	10-13	13-16	6-16	16-21
I	0	40	4.9 (1.2)[b]	3.9 (1.3)	5.2 (1.9)	7.8 (2.2)	5.5 (0.9)	17.7 (2.4)
II	0.05	19	5.0 (1.2)	2.9* (1.4)	5.0 (1.9)	6.3 (2.6)	4.5* (1.2)	16.7 (3.5)
III	0.10	20	4.5 (1.1)	2.2* (1.6)	4.3 (1.4)	6.9 (2.2)	4.2* (1.3)	16.7 (1.9)
IV	0.20	20	4.5 (1.2)	2.1* (1.5)	3.2* (2.3)	5.7* (2.4)	3.5* (1.1)	13.2* (2.1)

TREATMENT PERIOD

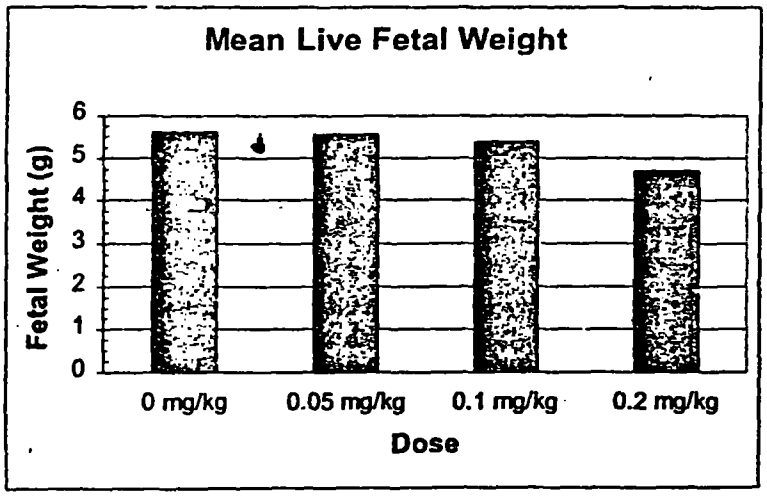
[a]Number of dams with viable fetuses.

[b]Figures in parentheses represent the sample standard deviations.

*Significantly different from controls, $p \leq 0.05$.

- Food consumption: Decreased food consumption at 0.1 and 0.2 mg/kg
- Toxicokinetics: Not done
- Embryo-fetal Development
  - In-life observations:
  - Terminal and Necroscopic evaluations:
    - Dams: No effects on corpora lutea, resorptions, nidation
- Offspring:

Fetal weight decreased 4 and 16% at 0.1 mg/kg and 0.2 mg/kg, respectively



Two fetuses with severe abnormalities were found at 0.05 mg/kg, however these abnormalities were not observed in at 0.1 or 0.2 mg/kg. All skeletal findings can be classified either common variant or minor anomalies. Retarded

development of the fetal kidney, as indicated by absence or small size of the renal papilla, occurred with greater frequency in drug treated groups (27-50% in treated fetuses versus 17% in controls fetuses)

Summary and Evaluation:

1. Treatment with mitoxantrone caused dose dependent decrease in body weight gain in the dams. The decreases were 18, 24 and 37 percent less than controls at 0.05, 0.1, and 0.2 mg/kg/day, respectively.
2. Decreased mean fetal weight was observed 0.1 and 0.2 mg/kg. This decrease was attributed to maternal toxicity;
3. Retarded development of the fetal kidney was observed at 0.05 mg/kg/day and above..
4. Mitoxantrone had no apparent teratogenic activity in this study.

A Teratology Study (Segment II) of CL 232,315 Administered Intravenously to Pregnant Rabbits (Study 147)

Study No: and number:81238
Amendment #, Volume # and Page #: Volume 31, Page 179-259
Site and testing facility: Lederle Laboratories, American Cyanamid Co, Pearl River, NY
GRP compliance: No
QA- Reports Yes (X) No ( ):
Lot and batch numbers: PC 0345
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Rabbit, New Zealand White

Group	Compound	Daily Dose[a] (mg/kg)	Inseminated Females
I	Vehicle[b]	...	18
II	CL 232,315	0.01	18
III	CL 232,315	0.025	18
IV	CL 232,315	0.05	18

[a] Dosages expressed as pure parent compound (free base). Animals were dosed on days 6-18 of pregnancy at a dosing volume of 1 mg/kg of body weight. Doses were adjusted on day 12 of pregnancy.

[b] Normal Saline, 0.9% (Sodium Chloride Injection, U.S.P.)

- Study Design:

Procedure	Subject	Day(s) of Pregnancy or Gestation
Selection	Females	Prior to start of inseminations
Randomization	Females	Following selection
Preinsemination examination	Females	0
Artificial insemination	Females	0
Body weight measurement	Females Fetuses	0, 6, 12, 18, 24, 30 30
Food consumption measurement	Females	6, 12, 18, 24, 30
Compound preparation	...	Daily 6-18 from stock solutions prepared on the first day of dosing
Compound administration	Females	6-18
Adjustment of dose	...	12
Clinical observations	Females	0, 6-18, 24, 30 and additionally as required
Sacrifice and postmortem observations	Females	30
Gross external and soft tissue examinations	Fetuses	30

- Statistical evaluations:

Results:

- Clinical signs:

0.05 mg/kg 2 cases of blood in urine, 0.01 mg/kg 1 case of blood in urine; rabbits delivered litters prematurely three days after observation.

Ear lesions were observed at the injection sites; incidence and severity of the lesions increased with dose.

- Mortality: Two rabbits at 0.025 mg/kg and one control rabbit died of respiratory infections; no deaths attributed to treatment
- Body weight: No treatment related effects on body weight
- Food consumption: No treatment related effects on food consumption
- Toxicokinetics: Not done
- Fertility and Early Embryonic Development in Females
- In-life observations

	0 mg/kg	0.01 mg/kg	0.025 mg/kg	0.05 mg/kg
Premature Delivery	1/15	4/16	2/14	5/17

- Terminal and Necroscopic evaluations:

Five fetuses with major malformations were seen (two in 0.025 mg/kg and control groups, one in 0.05 mg/kg) group. No significant increase in any single malformations or variations

Summary and Evaluation:

1. Clinical signs observed in rabbits included hematuria; No effects were observed on body weight, which suggests that the dose might have been higher, however a range finding suggested that a 0.25 mg/kg would be lethal to rabbits. Considering the increased incidence of premature delivery, the doses were probably adequate.
2. An increase incidence of premature delivery was observed with mitoxantrone treatment at all doses.
3. Three rabbits died of respiratory infections during this study.
4. No significant effects on the embryo or fetus were observed in this study.

Labeling Recommendations:

Summary

Mitoxantrone was tested for reproductive toxicity in a combination Segment I/III study and two Segment II studies (in rats and rabbits). In all these studies, the dose mitoxantrone was high enough to cause slight toxicity (neutropenia, decreased weight gain, premature delivery). In general, the adverse effects observed were those associated with effects on the parental generation rather than on the embryo or fetus. Adverse effects observed included:

1. Decreased maternal weight gain (associated with decreased fetal weight) at 0.1 mg/kg/day (0.6 mg/m^2/day) from gestation days 6 through 16 in rats;
2. Retarded development of the fetal kidney was observed at 0.05 mg/kg/day (0.3 mg/m^2/day) and above from gestation days 6 through 16 in rats.
3. Premature delivery of litters at 0.01 mg/kg/day (0.12 mg/m^2/day) from gestation days 6 through 18 in rabbits only;
4. Decreased litter size in female rats given 0.03 mg/kg/day (0.18 mg/m^2/day) from 21 days premating through delivery and male rats dosed for 71 days prior to mating.

No effects were observed on pup development or fertility in the two generation study.

Since mitoxantrone is genotoxic in a variety of assays (see below), and since it affects reproductive performance at doses below the clinical dose, it is recommended that Pregnancy Category D be retained for this drug.

GENETIC TOXICOLOGY:

Microbial Mutagenicity

Microbial mutagenicity test on CL 232,315 (Report 127)

Study No:E167M
Study Type: Microbial Mutagenicity
Amendment #, Volume # and Page #: Volume 28, Page 136-154
Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY
Date of Study Initiation/completion: 2/1/1978 to 3/13/1978
GLP Compliance: No
QA- Reports Yes ( ) No ( ):
Drug Lot Number: 10987B-93A
Study Endpoint: Microbial mutagenesis

Methodology:

- Strains/Species/Cell line: *Salmonella typhimurium* TA1535, TA100, TA1537, TA98, TA1538, *Escherichia coli* wp-2 uvrA
- Dose Selection Criteria:
  - Basis of dose selection: noncytotoxic doses
  - Range finding studies: Yes
- Test Agent Stability:
- Metabolic Activation System: Rat S9
- Controls:
  - Vehicle: Water
  - Negative Controls: Water, DMSO
  - Positive Controls: MNNG (TA1535, TA100 –S9), 2-Aminofluorene (TA1538, TA98+S9), 9-aminoacridine (TA1537)
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: 48 hours
  - Doses used in definitive study: 0.1 to 1000 ug/plate
  - Study design:
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 3 replicates/dose
  - Counting method:
  - Cytotoxic endpoints: decreased bacterial lawn
  - Genetic toxicity endpoints/results: revertant colonies
  - Statistical methods:
- Other:
- Criteria for Positive Results: Doubling of revertant colonies

Results:

- Study Validity:
- Study Outcome:

Strain	Mutation	-S9	+S9
TA98	**Frame Shift**	**Positive at 100 ug/plate**	**Positive at 100 ug/plate**
TA100	GC Base Pair	Cytotoxic at 100 ug/plate	Cytotoxic at 1000 ug/plate
TA1535	GC Base Pair	Cytotoxic at 100 ug/plate	Cytotoxic at 100 ug/plate
TA1537	**Frame Shift**	Cytotoxic at 10 ug/plate	**Positive at 10 ug/plate**
TA1538	**Frame Shift**	**Positive at 10 ug/plate**	**Positive at 10 ug/plate**
E coli	AT Base Pair	Cytotoxic at 100 ug/plate	Cytotoxic at 1000 ug/plate

Microbial mutagenicity test on CL 232,315 (Report 128)

Study No:E167M
Study Type: Microbial Mutagenicity
Amendment #, Volume # and Page #: Volume 28, Page 155-182
Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River
Date of Study Initiation/completion: 5/25/1982 to 5/27/1982
GLP Compliance: No
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0345
Study Endpoint: Microbial mutagenesis

Methodology:

- Strains/Species/Cell line: *Salmonella typhimurium* TA1535, TA100, TA1537, TA98, TA1538, *Escherichia coli* wp-2 uvrA
- Dose Selection Criteria:
  - Basis of dose selection: noncytotoxic doses
  - Range finding studies: Yes
- Test Agent Stability: Yes
- Metabolic Activation System: Rat S9
- Controls:
  - Vehicle: Water
  - Negative Controls: Water, DMSO
- Positive Controls:

Strain	Mutation	-S9	+S9
TA98	Frame Shift	20 ug 2-Nitrofluorene	5 ug 2-Aminoanthracene
TA100	GC Base Pair	10 ug N-methyl-N'-nitro-N-Nitrosoguanidine	5 ug 2-Aminoanthracene
TA1535	GC Base Pair	10 ug N-methyl-N'-nitro-N-Nitrosoguanidine	5 ug 2-Aminoanthracene
TA1537	Frame Shift	50 ug 9-Aminoacridine	5 ug 2-Aminoanthracene
TA1538	Frame Shift	20 ug 2-Nitrofluorene	5 ug 2-Aminoanthracene
E coli	AT Base Pair	10 ug N-methyl-N'-nitro-N-Nitrosoguanidine	Same as –S9

- Comments:
- Exposure Conditions:
  - Incubation and sampling times: 48 hours
  - Doses used in definitive study: 12, 60, 300, 600 ug/plate
  - Study design:
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 3 replicates/dose
  - Counting method:
  - Cytotoxic endpoints: decreased bacterial lawn
  - Genetic toxicity endpoints/results: revertant colonies
  - Statistical methods:
- Other:
- Criteria for Positive Results: Doubling of revertant colonies

Results:

- Study Validity:
- Study Outcome:

Strain	Mutation	-S9	+S9
TA98	**Frame Shift**	**Positive at 50 ug/plate**	**Positive at 50 ug/plate**
TA100	GC Base Pair	Negative at 500 ug/plate	Negative at 500 ug/plate
TA1535	GC Base Pair	Negative at 500 ug/plate	Negative at 500 ug/plate
TA1537	**Frame Shift**	**Positive at 50 ug/plate**	**Positive at 50 ug/plate**
TA1538	**Frame Shift**	**Positive at 10 ug/plate**	**Positive at 10 ug/plate**
E coli	AT Base Pair	Negative at 500 ug/plate	Negative at 500 ug/plate

In Vitro Mammalian DNA Damage and Mutagenicity

The Unscheduled DNA Synthesis (UDS) Test on CL 232,315 (Mitoxantrone Hydrochloride) Using Primary Rat Hepatocytes in Culture (Report 130)

Volume
Study No: 81285
Study Type: DNA Damage in vitro
Amendment #, Volume # and Page #: Volume 28, Page 187-233
Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY
Date of Study Initiation/completion: 11/17/1981 to 12/29/1981
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0345
Study Endpoint: Tritiated thymidine incorporation in DNA

Methodology:

- Strains/Species/Cell line: Freshly isolate rat hepatocytes from male Crl:COBS(SD)CD strain
- Dose Selection Criteria:
  - Basis of dose selection:
  - Range finding studies:
- Test Agent Stability: Yes
- Metabolic Activation System:
- Controls: See table
- Exposure Conditions:
  - Incubation and sampling times: 18-20 hour incubation
  - Doses used in definitive study: see table
- Study design:

Group	Treatment	Concentration mcg/well[b]	Concentration mcg/mL	Number of Replicates
1	Vehicle[c]	0	0	3
2	CL 232,315	0.003	0.001	3
3	CL 232,315	0.015	0.005	3
4	CL 232,315	0.030	0.010	3
5	CL 232,315	0.15	0.05	3
6	CL 232,315	0.30	0.10	3
7	CL 232,315	1.5	0.50	3
8	CL 232,315	3.0	1.0	3
9	CL 232,315	6.0	2.0	3
10	CL 878[d]	2.7	0.9	3
11	CL 30,205[e]	4.0	1.3	3

[a] Cultures initiated November 17, 1981. Treatment with test and reference substance began November 17, 1981 and ended November 18, 1981.
[b] Each well contained 3 mL of culture medium.
[c] Normal Saline, U.S.P.
[d] 2-Aminofluorene (2-AF) in dimethylsulfoxide (DMSO).
[e] N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) in DMSO.

APPEARS THIS WAY ON ORIGINAL

- Analysis:
  - No. slides/plates/replicates/animals analyzed:
  - Counting method:

- Cytotoxic endpoints: viable cells / field
- Genetic toxicity endpoints/results: grains/nucleus
- Statistical methods:

- Other:
- Criteria for Positive Results: 6 grains/nucleus above control

Results:

- Study Validity: Positive controls were positive and negative controls were negative
- Study Outcome

Group	Treatment	Concentration[b] mcg/mL	Mean Net Nuclear[c] Grains (± S.D.)	Toxicity
1	Vehicle[d]	0	4.56 (± 4.58)	—
2	CL 232,315	0.001	7.61 (± 7.86)	Not Toxic
3	CL 232,315	0.005	8.30 (± 7.77)	Not Toxic
4	CL 232,315	0.010	6.67 (± 7.74)	Toxic
5	CL 232,315	0.050	9.23 (± 9.82)	Toxic
6	CL 232,315	0.100	10.20 (± 8.40)	Toxic
7	CL 232,315	0.500	15.44 (±10.69)	Toxic
8	CL 232,315	1.00	19.81 (±13.25)	Toxic
9	CL 232,315	2.00	e	Toxic
10	CL 878[f]	0.9	78.62 (±32.64)	Not Toxic
11	CL 30,205[g]	1.3	97.72 (±43.11)	Not Toxic

[a] Cultures initiated November 17, 1981. Treatment with test and reference substances began November 17, 1981 and ended November 18, 1981.
[b] Each well contained 3.0 mL of culture medium.
[c] A total of 150 cells were counted per dose group.
[d] Normal Saline, U.S.P.
[e] No viable cells.
[f] 2-Aminofluorene (2-AF) in dimethylsulfoxide (DMSO).
[g] N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) in DMSO.

Unscheduled DNA synthesis was induced at all dose levels tested (0.001 to 1 ug/ml).

APPEARS THIS WAY
ON ORIGINAL

Sister Chromatid Exchange (SCE) Analysis on CL 232,315 Using Chinese Hamster Ovary (CHO) Cells (Report 131)

Study No:83311
Study Type: Sister Chromatid Exchange
Amendment #, Volume # and Page #: Volume 29, Pages 1-28
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 10/17/1983 to 1/23/1984
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0345
Study Endpoint: Clastogenesis/in vitro

Methodology:

- Strains/Species/Cell line: CHO Cells
- Dose Selection Criteria:
  - Basis of dose selection: reduced mitotic index
  - Range finding studies: yes
- Test Agent Stability: Yes
- Metabolic Activation System: Rat S9
- Controls:
  - Vehicle: 0.9% saline
  - Negative Controls:
  - Positive Controls: 0.025 ug/ml triethylenemelamine; 2.5 ug/ml cyclophosphamide
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: 24 hours (-S9); 2 hours (+S9)
  - Doses used in definitive study: 0.31, 0.625, 1.25, 2.5, 5, 10 ng/ml (-S9)
    0.03, 0.1, 0.3, 1, 3, 10 (+S9)
  - Study design: blinded analysis
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 2 replicate/dose
  - Counting method: 25 cells/culture
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results: SCE/cell
  - Statistical methods: student t-test
- Other:
- Criteria for Positive Results: doubling of SCE at 2 dose levels, t-test, dose response over three points

Results:

- Study Validity:positive controls were positive and negative control was negative
- Study Outcome- positive as 1 ng/ml and above with S9 and 2.5 ng/ml and above without S9

Summary:

APPEARS THIS WAY
ON ORIGINAL

Mammalian Mutagenesis Assay on CL 232,315 Using the L5178Y TK+/- Mouse Lymphoma System (Study 133)

Study No:83303
Study Type: Mammalian cell mutations
Amendment #, Volume # and Page #: Volume 29, Pages 39-91
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 10/18/1983 to 1/17/1984
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number:PC 0345
Study Endpoint: Mutations

APPEARS THIS WAY ON ORIGINAL

Methodology:

- Strains/Species/Cell line: L5178Y TK+/- lymphoma cells
- Dose Selection Criteria:
  - Basis of dose selection: cytotoxicity cell population density
  - Range finding studies:Yes
- Test Agent Stability: Stable
- Metabolic Activation System: Rat S9
- Controls:
  - Vehicle: DMSO
  - Negative Controls: Yes
  - Positive Controls: 0.5, 1 ul/ml Ethylmethanesulfonate, 5, 7.5 ug/ml 7,12-Dimethylbenzanthracene
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: 4 hours
  - Doses used in definitive study: 0.3, 0.7, 1.1, 1.5 ng/ml (-S9), 30, 70, 110, 150 ng/ml (+S9)
  - Study design:
- Analysis:
  - No. slides/plates/replicates/animals analyzed:
  - Counting method:
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results:
  - Statistical methods:
- Other:
- Criteria for Positive Results:

Positive - if there is a positive dose response and one or more of the three highest doses exhibit a mutant frequency which is two-fold greater than the background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Results:

- Study Validity: positive controls positive, negative controls negative
- Study Outcome: Mitoxantrone was positive at all dose levels tested, 0.3 ng/ml and above without metabolic activation and 30 ng/ml and above with metabolic activation

Cell Transformation Assay on CL 232,315 and CL 115,751 (Adriamycin) Using $C_3H/10T1/2$ CL 8 Cell Line in Culture (Study 134)

Study No:83325
Study Type: Clastogenesis in vitro
Amendment #, Volume # and Page #: Volume 29, Pages 92-120
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 11/1/1983 to 1/10/1984
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0345
Study Endpoint: Clastogenesis in vitro

Methodology:

- Strains/Species/Cell line: $C_3H/10T1/2$ CL 8 Cell Line
- Dose Selection Criteria:
  - Basis of dose selection: cytotoxicity
  - Range finding studies: yes
- Test Agent Stability:
- Metabolic Activation System:
- Controls:
  - Vehicle:
  - Negative Controls:10% (v/v) 0.9% NaCl
  - Positive Controls: 2.5, 5, 10 ug/ml 3-methylcholanthrene
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: 24 hours, scored 6 weeks post exposure
  - Doses used in definitive study: 0.031, 0.63, 0.125, 0.25, 0.5 ng/ml
  - Study design:
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 15-16 cultures/dose level
  - Counting method: Giemsa staining
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results: transformed colonies
  - Statistical methods: Katenbaum and Bowman tables
- Other:
- Criteria for Positive Results:

To determine whether the results at each dose level constitute a positive response in this assay, 1) The statistical tables of Kastenbaum and Bowman (1970) will be used to determine if the results at each dose level are significantly different from the negative control at the 99% and/or 95% confidence levels and 2) The absolute transformation frequencies in the treated dishes will be compared to the negative control frequencies to determine the ratio of the transformation frequencies in the treated dishes at each dose to the negative control frequency. A response at a particular dose level that attains the 95% confidence level or that results in a treated/negative control frequency ratio of 2.0 or higher will usually provide evidence for classifying a test article as active.

Results:

- Study Validity: positive controls positive, negative controls negative
- Study Outcome No significant increase in transformed colonies was observed.

APPEARS THIS WAY ON ORIGINAL

In Vivo Mammalian DNA Damage

An In Vivo Cytogenetic Study of CL 232,315 Administered Intraperitoneally to Male Rats Once Daily for 5 consecutive Days (Report 138)

Study Type: Clastogenesis in vivo
Amendment #, Volume # and Page #: Volume 29, Pages 138-165
Conducting Laboratory: Lederle Laboratories, Pearl River, NY
Date of Study Initiation/completion:
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0338
Study Endpoint:

APPEARS THIS WAY ON ORIGINAL

Methodology:

- Strains/Species/Cell line: Male Rats Crl:COBS CD (SD)
- Dose Selection Criteria:
  - Basis of dose selection: Toxicity to rats
  - Range finding studies: Yes
- Test Agent Stability: Yes
- Metabolic Activation System: N/A
- Study design:

Group	Compound	Dose[a] mg/kg	Dose[a] mg/m^2	Number of Days[b]	Route of Administration	Number of Animals
1	Vehicle[c]			5	Intraperitoneally	10
2	CL 232,315	0.5	3.6	5	Intraperitoneally	10
3	CL 232,315	1.0	7.1	5	Intraperitoneally	10
4	CL 232,315	2.0	14.2	5	Intraperitoneally	10
5	CL 5975[d]	0.3		5	Intraperitoneally	10

[a]Dose is given in terms of pure parent compound. One gram of pure parent compound was contained in 1.4 g of compound as weighed. The dosage for this compound is expressed both as mg/kg and as mg/m^2. For rats, the factor for converting dose in mg/kg to dose in mg/m^2 is derived as follows:[4]

$$\frac{(\text{Mean body weight in kg})^{1/3} \times 100}{9}$$

For this study, the factor for the rats dosed with CL 232,315 was:

$$\frac{(.263)^{1/3} \times 100}{9} = 7.1$$

[b]The first dose was administered on test Day 0, November 28, 1979.

[c]The vehicle for both compounds was 0.9% NaCl.

[d]Reference compound, triethylenemelamine.

- Analysis:
  - No. slides/plates/replicates/animals analyzed: 100 metaphases/rat
  - Counting method:
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results: chromosomal aberrations in bone marrow cells
  - Statistical methods: t-test, transformed to the arc sine of the square root
- Other:
- Criteria for Positive Results:

Results:

Number and Type of Aberrations Observed in a Sample of Selected Diploid Cells

Group:	1	2	3	4	5
Compound:	Saline	CL 232,315	CL 232,315	CL 232,315	CL 5975†
Dose (mg/kg/day):		0.5	1.0	2.0	0.3
Number of Animals*	10	10	9	5	10
Total Metaphases**	950	999	585	277	630
Metaphases with Aberrations	4	16	32	54	58
Type of Aberration					
Chromatid Break	1	5	7	7	6
Fragment	3	8	12	8	22
Chromosome Break			1		4
Tri- or Quadriradial		2	1	1	2
Ring					1
Multiple		1	9	20	8
Complex			2	18	15

*Those rats with fewer than 10 acceptable metaphases were not included.

**The number of metaphases from each rat varied. See Appendix IV for the number sampled from each rat.

†Reference compound, triethylenemelamine.

Mitoxantrone induced chromosomal aberrations in rats starting at 0.5 mg/kg/day for 5 days.

An In Vivo Cytogenetic Study on the Effect of CL 232,315 Administered Intravenously to Male Rats at 21 Day Intervals (Report 139)

Study No: 81107
Study Type: Clastogenesis in vivo
Amendment #, Volume # and Page #: Volume 29, Pages 166-205
Conducting Laboratory: Lederle Laboratories, Pearl River, NY
Date of Study Initiation/completion: 5/27/1981 to 8/21/1981
GLP Compliance:
QA- Reports Yes (X) No ( ):
Drug Lot Number: PC 0345
Study Endpoint:

Methodology:

- Strains/Species/Cell line: Male Rats Crl:COBS CD (SD)
- Dose Selection Criteria:
  - Basis of dose selection: Toxicity to rats
  - Range finding studies: Yes
- Test Agent Stability: Yes
- Metabolic Activation System: N/A
- Study design:

Experimental Design

Group	Treatment	Dose[a] mg/kg	Route of Administration	No. of Animals[b] Treated Day 0	Treated Day 21	Sacrifice Day 1	Sacrifice Day 21	Sacrifice Day 42
1	Vehicle[c]	0	Intravenous	30	10[d]	10	10	10
2	CL 232,315	0.3	Intravenous	30	10	10	10	10
3	CL 5975[e]	0.3	Intraperitoneal	10	-	10	-	-

[a] Administered at a dose volume of 1 mL/100 g body weight
[b] Study Day 0 = May 27, 1981, the date of administration of the first dose
[c] 0.9% sterile saline
[d] All animals receiving a second dose on Day 21 were sacrificed on Day 42
[e] Reference compound, triethylenemelamine, in 0.9% sterile saline

- Analysis:
  - No. slides/plates/replicates/animals analyzed: 100 metaphases/rat
  - Counting method:
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results: chromosomal aberrations in bone marrow cells
  - Statistical methods: t-test, transformed to the arc sine of the square root
- Other:
- Criteria for Positive Results:

Results:

Number and Type of Aberrations Observed in a Sample of Selected Diploid Cells

Treatment	0.9% Saline	0.9% Saline	0.9% Saline	CL 232,315	CL 232,315	CL 232,315	CL 9975*
Dose Administration (Study Day)	0	0	0, 21	0	0	0, 21	0
Dose (mg/kg/day)†	-	-	-	0.3	0.3	0.3	0.3
Sacrifice (Study Day)	1	21	42	1	21	42	1
Number of Animals	10	10	10	10	10	10	10
Total Metaphases**	1000	1000	1000	1000	1000	1000	791
Metaphases with Aberrations	4	7	0	33	12	3	242
Type of Aberration							
Chromatid Break	3	0	0	12	6	2	22
Fragment	1	6	0	10	5	1	14
Chromosome Break	0	1	0	3	1	0	5
Tri- or Quadriradial	0	0	0	0	0	0	5
Ring	0	0	0	0	0	0	0
Multiple	0	0	0	6	0	0	95
Complex	0	0	0	2	0	0	101

*Reference compound, triethylenemelamine, administered intraperitoneally.

†Administered at a dose of 1.0 ml/100 g body weight.

**Includes all readable metaphases up to a maximum of 100 per rat.

Mitoxantrone induced chromosomal aberrations 24 hours after a single IV injection at the clinical dose. The significance of the return to near control levels of chromosomal aberrations is uncertain due to the rapid turnover of cells in the bone marrow.

Dominant Lethal Test on CL 232,315 Administered Intraperitoneally in Rats (Reports 141, 142)

Study No:E351M, E3519
Study Type: Dominant Lethal
Amendment #, Volume # and Page #: Volume 29, Pages 215-265
Conducting Laboratory: Lederle Laboratories, Pearl River, NY
Date of Study Initiation/completion: 6/1/1978 to 10/1/1978
GLP Compliance:
QA- Reports Yes ( ) No (X):
Drug Lot Number: 10987B-93A
Study Endpoint:

Methodology:

- Strains/Species/Cell line: Male Rat, Crl:COBS CD (SD)
- Dose Selection Criteria:
  - Basis of dose selection:
  - Range finding studies:
- Test Agent Stability: N/A
- Metabolic Activation System: N/A
- Controls:
  - Vehicle: saline
  - Negative Controls: Saline
  - Positive Controls: 0.05 mg/kg/day triethylenemelamine
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times:
  - Doses used in definitive study: 0.5, 1, 2 mg/kg/day for 5days
  - Study design: rats mated 5 days/week for 8 weeks, different female each week; pregnant dams sacrificed on Day 13 of gestation and uterus examined for implantations and resorptions
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 10 rats/dose
  - Counting method:
  - Cytotoxic endpoints:
  - Genetic toxicity endpoints/results:
  - Statistical methods:
- Other:
- Criteria for Positive Results:

Results:

- Study Validity:
- Study Outcome: decreased fertility at 2 mg/kg; no effects on implantations or embryo survival

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Summary of Genotoxicity

Mitoxantrone is genotoxic in a variety of assays. In bacterial test systems, it induces frame shift mutations in the Ames assay, although it did not increase the incidence of point mutations. It also induced mutations in the mouse lymphoma assay. Mitoxantrone also damaged DNA in in vivo and in vitro assays.

System	Endpoint	In Vivo/ In Vitro	Lowest Positive Dose	Highest Negative Dose
Ames Test	Mutations	In Vitro	10 ug/plate	1 ug/plate
Mouse Lymphoma	Mutations	In Vitro	0.3 ng/ml	---
Unscheduled DNA Synthesis	DNA Damage	In Vitro	1 ng/ml	---
Sister Chromatid Exchange	DNA Damage	In Vitro	1 ng/ml	0.3 ng/ml
Cell Transformation	DNA Damage	In Vitro	---	0.5 ng/ml
Bone Marrow Cytogenetics	DNA Damage	In Vivo	0.3 mg/kg	---
Dominant Lethal	DNA Damage/ Mutation	In Vivo	---	2 mg/kg

OVERALL SUMMARY AND EVALUATION:

Introduction:

Mitoxantrone is a cytotoxic agent which has been proposed for use in multiple sclerosis patients. The proposed clinical dose is 14 mg/m^2 every three months. Mitoxantrone is already approved for use in patients with hormone-refractory prostate cancer and acute nonlymphocytic leukemia. In cancer patients, the clinical dose is 12-14 mg/m^2 every three weeks.

Mitoxantrone exerts its cellular effects by interfering with DNA structure and function. It intercalates into DNA causing DNA crosslinks and DNA double- and single-strand breaks. Mitoxantrone also interferes with RNA molecules in the cell nucleus. In addition, mitoxantrone is a potent inhibitor of topoisomerase II, an enzyme responsible for uncoiling and repairing damaged DNA. Due to the sensitivity of the immune system to agents which interfere with DNA structure and function, mitoxantrone is an immunosuppressive agent.

Since multiple sclerosis is thought to be caused in part by autoimmunity, it thought that immunosuppressive agents would be beneficial in these cases. Mitoxantrone prevented or treated an animal model of multiple sclerosis, experimental allergic encephalomyelitis. In addition, mitoxantrone inhibits the activity of T-cells, B-cells and macrophages.

Safety Evaluation:

Pharmacokinetics

The pharmacokinetic studies have been conducted with radiolabelled mitoxantrone, which does not distinguish between parent compound and metabolites. Since mitoxantrone is administered intravenously, absorption is complete. After administration, mitxantrone and/or its metabolites are extensively distributed to the tissues; the apparent volume of distribution in rats was 392 l/kg. The radiolabelled mitoxantrone is slowly eliminated from the body; the half life in rats is 12 days, although it is not possible to assess whether mitoxantrone is present unchanged or as one of its metabolites. The major route of excretion is via the bile, generally as a mono- or dicarboxy derivative and their glucorinide metabolites. The urine is another significant route of excretion.

Acute Toxicity

A listing of the lowest lethal intravenous doses and the highest nonlethal dose in various species is presented in the following table. The lowest lethal doses are consistent across species and range from 0.5 mg/kg in the dog to 7 mg/kg in mice, a factor of 14; when doses are expressed as mg/m^2, the range is from 10 to 21, a factor of two. The signs of toxicity were consistent across species and doses and included diarrhea, epistaxis (rats and monkeys), emesis (dogs and monkeys only), edema, hypothermia (rats and dogs), and weight loss. At the lower range of lethality, the time between exposure and death was often more than seven days. Since the proposed clinical dose is 12 mg/m^2, it is apparent that mitoxantrone has a narrow therapeutic index in humans.

Species	Lethal Dose (mg/kg)	Lethal Dose (mg/m^2)	Nonlethal Dose (mg/kg)	Nonlethal Dose (mg/m^2)
Mouse	7	21	5.5	16.5
Rat	3.0	18	1	6
Dog	0.5	10	0.375	7.5
Monkey	1	18	0.5	9

The primary toxicity observed during these studies was effects on the lymphoreticular tissues accompanied by leukopenia and anemia. The leukopenia was more severe than the anemia and was characterized by decreased lymphocytes and neutrophils. The nadir of the white blood cell counts generally occurred about 10 to 11 days after injections. At doses comparable to the proposed human clinical dose (12 mg/m^2), mitoxantrone caused a 43%, 84%, and 88% decrease in white blood cell counts in rats (6 mg/m^2), dogs (10 mg/m^2), and monkeys (12 mg/m^2), respectively. In comparison, the red blood cell counts were depressed 7%, 19%, and 47% in rats, dogs and monkeys at the same doses. Bone marrow hypocellularity was observed at 0.3 mg/kg and above in rats, 1 mg/kg and above in dogs, and 3 mg/kg in monkeys. In addition, rats administered 1 mg/kg mitoxantrone had increased fat tissue in the bone marrow. Besides the effects on the bone marrow, there was lymphoid depletion in the thymus, spleen and

lymph nodes. A summary of the Lowest Effect Levels and Highest No Effect Levels for leukopenia and anemia is presented below.

Lowest Effect Level (Highest No Effect Level) for Mitoxantrone in Various Species

	Rat		Dog		Monkey	
	Mg/kg	Mg/m^2	Mg/kg	Mg/m^2	Mg/kg	Mg/m^2
Leukopenia	0.03 (N/A)	0.18 (N/A)	0.1875 (N/A)	3.75 (N/A)	0.25 (N/A)	4.5 (N/A)
Anemia	3 (1)	18 (6)	1 (0.5)	20 (10)	1 (0.5)	18 (9)

The kidney is a significant target organ in these studies. In rats, 3 mg/kg caused kidney failure characterized by hematuria, increased BUN, anemia, and kidney changes (hydropic degeneration, interstitial tissue proliferation with round cell infiltration, and fibrosis) 42 days post dosing. Slight to moderate hydropic degeneration of the kidney was observed at 1 mg/kg 21 and 42 post dosing. Increased relative kidney weight was observed at 1 mg/kg in rats. Related clinical pathology signs of kidney dysfunction included increased serum cholesterol and triglycerides. In dogs, increased BUN was observed at 1 mg/kg and above. In addition, the kidneys were hyperemic at 1 mg/kg and congested at 4 mg/kg. In monkeys, perivascular inflammation of the kidneys was observed at 1 mg/kg.

The heart and cardiovascular system is a target organ for mitoxantrone. Heart hemorrhages were observed at 0.5 mg/kg in dogs. Hydropericardium was observed at 1 mg/kg.

The intestines are target organs in dogs and monkeys. In dogs, colitis, duodenal hemorrhage and bloody diarrhea were observed at 4 mg/kg. Large intestine hemorrhage and ulceration was observed at 0.5 mg/kg in dogs. In monkeys, enteritis was observed at 3 mg/kg and above.

Chronic Toxicity

The chronic toxicity of mitoxantrone has been studied in rats, dogs, monkeys and rabbits. The rats, dogs and monkeys were dosed at three week intervals to mimic the clinical exposure, but the rabbits were dosed at one week intervals. In general, the toxic effects observed in the chronic studies are similar to those in the acute studies. The hematopoietic system was the primary target organ of concern. However the heart, kidney, and testes are additional target organs of concern. Neoplasms were also observed in the rat chronic study (see Carcinogenicity section below).

Mitoxantrone caused substantial early mortality in chronic studies at doses below the proposed clinical dose of 12 mg/m^2. In rats, substantial early mortality (100% in males, 45% in females) was observed in rats administered 0.3 mg/kg (1.8 mg/m^2). The mortality was associated with the cumulative dose reaching 4.2 to 4.4 mg/kg (25.2 mg/m^2 to 26.4 mg/m^2) in males and 6.5 to 6.7 mg/kg (39.0 mg/m^2 to 40.2 mg/m^2) in females. In dogs, one out of six dogs administered 0.25 mg/kg (5.15 mg/m^2) every three weeks over 30 weeks died. In monkeys, three out of ten animals administered 0.25 mg/kg (3 mg/m^2) every three weeks over 44 week died. Rats were more vulnerable to mitoxantrone mortality than dogs or monkeys.

Neutropenia was the most striking toxic effect of mitoxantrone. In the dog and monkey study, where it was possible to do serial white blood cell counts, the graph of the white blood cell counts with time formed a "saw toothed" pattern with a nadir in the count at post dosing day 11 followed by recovery to near pre dose levels just prior to the next dose. At high doses (0.25 mg/kg in dogs), nadir became lower with time, but at lower doses (e.g. 0.125 in dogs or 0.25 mg/kg in monkeys) there appeared to be less cumulative effect. Red blood cell counts were also depressed, although to a lesser extent than white blood cell counts. Hypocellularity of the bone marrow was often observed in neutropenic animals. In addition, lymphoid depletion of the thymus, spleen and lymph nodes were frequently observed.

The heart is another target organ in rats, monkeys, and rabbits. Effects included heart muscle necrosis in rats at 0.3 mg/kg, interstitial fibrosis in monkeys at 0.125 mg/kg and above and myocardial changes (including focal necrosis and thrombi), fibrosis and fiber vauolation in rabbits at 0.125 mg/kg. In the two year carcinogenicity study in rats, an increased incidence of heart mineralization was observed at 0.1 mg/kg.

Mitoxantrone also affects the kidneys. In the two year carcinogenicity study in rats, glomulonephritis was the primary cause of premature death in male rats at 0.1 mg/kg every three weeks. In the twelve month rat study, nephrosclerosis was observed at 0.03 mg/kg in males and 0.3 mg/kg in females; hydropic changes with proximal tubule necrosis was observed at 0.3 mg/kg and above; urinalysis alterations at 0.3 mg/kg included proteinuria, occult blood and increased urine volume.

The testes were affected by mitoxantrone treatment. At 0.125 mg/kg diffuse tubular atrophy of the testes and aspermia of the epidiymis was observe in dogs. The testes weight was decreased by about 50 percent in these dogs. In monkeys administered 0.25 mg/kg, decreased spermatogenesis in the testes and oligospermia in the epididymis were observerd. Finally, testitcular tubule atrophy was observed in rabbits at 0.125 mg/kg.

Genotoxicity

Mitoxantrone is genotoxic in a variety of assays. In bacterial test systems, it induces frame shift mutations in the Ames assay, although it did not increase the incidence of point mutations. It also induced mutations in the mouse lymphoma assay. Mitoxantrone also damaged DNA in in vivo and in vitro assays.

System	Endpoint	In Vivo/ In Vitro	Lowest Positive Dose	Highest Negative Dose
Ames Test	Mutations	In Vitro	10 ug/plate	1 ug/plate
Mouse Lymphoma	Mutations	In Vitro	0.3 ng/ml	---
Unscheduled DNA Synthesis	DNA Damage	In Vitro	1 ng/ml	---
Sister Chromatid Exchange	DNA Damage	In Vitro	1 ng/ml	0.3 ng/ml
Cell Transformation	DNA Damage	In Vitro	---	0.5 ng/ml
Bone Marrow Cytogenetics	DNA Damage	In Vivo	0.3 mg/kg	---
Dominant Lethal	DNA Damage/ Mutation	In Vivo	---	2 mg/kg

Carcinogenicity

Mitoxantrone was tested for carcinogenicity in two mouse studies and one rat study. In the first mouse study, mice were administered 0.1, 0.2 and 0.4 mg/kg (0.3, 0.6, 1.2 mg/m^2) every 21 days. The 0.4 mg/kg dose exceeded the maximum tolerated dose causing substantial early mortality. No effects were observed on body weight and the white blood cell counts were not affected by mitoxantrone treatment. An increased incidence of hepatocellular adenoma was observed in male mice at a dose of 0.1 mg/kg (0.03 fold the recommended human dose, on a mg/m^2 basis). In the second carcinogenicity study, mice were administered 0.01, 0.03, and 0.06 mg/kg (0.03, 0.09, 0.18 mg/m^2) every 21 days. Mitoxantrone had no effects on mouse survival, body weight, or clinical condition. No carcinogenic effects were observed in this study, however the doses used in this study were below the maximum tolerated dose so that this study could not be considered an adequate test of carcinogenic potential.

In the rat study, rats were administered 0.01, 0.03 or 0.1 mg/kg (0.06, 0.18, or 0.6 mg/m^2) every 21 days. Increased mortality was observed 0.1 mg/kg in females and 003 and above in males. The early mortality was ascribed to increased incidence of glomularnephritis; an increased incidence of cardiac change was also observed in 0.1 mg/kg males. No significant effects were observed on white blood cell counts or other hematological parameters. An increased incidence of external auditory canal tumors and fibromas was observed at 0.03 mg/kg (0.02 fold the recommended human dose, on a mg/m^2 basis).

Results from other studies also suggest that mitoxantrone is carcinogenic in experimental animals. In a one year toxicity study, rats were administered 0.03, 0.3, 0.6, or 0.9 mg/kg (0.18, 1.8, 3.6, or 5.4 mg/m^2) every 21 days. Early mortality was observed 0.3 mg/kg and above; none of the males survived 44 weeks of treatment at these doses. At necropsy, 5/24 female rats in the 0.3 mg/kg group had auditory canal tumors. In addition, 1/17 female rats in the 0.6 mg/kg group had auditory canal tumors, despite none of these rats being on the study for more than 41 weeks. Finally, 1/18 male rats in the 0.3 mg/kg group had auditory canal tumors, despite none of these rats being on the study for more than 44 weeks. These results suggest that higher doses of mitoxantrone, which are still below the clinical dose (0.15 and 0.3 fold the clinical dose on a mg/m^2 basis), are carcinogenic in rats when administered for less than one year. In other studies, mitoxantrone is genotoxic in bacterial and mammalian test systems (both in vivo and in vitro). Mitoxantrone is a topoisomerase II inhibitor. Topoisomerase II inhibitors, in combination with other antineoplastic agents, have been associated with the development of acute leukemia in humans.

In summary, mitoxantrone is carcinogenic in two species of experimental animals at doses below the proposed clinical dose. Indeed, the carcinogenicity is expressed at doses which do not any signs of toxicity (eg neutropenia). In addition, mitoxantrone induce the same tumors observed in the 25 month carcinogenicity study in a 12 month toxicity study. This suggests that mitoxantrone is an active carcinogen in mice and rats. Mitoxantrone is also genotoxic in in vivo and in vitro systems.. Finally this class of compound (topoisomerase II inhibitors) are associated with leukemias in humans. Thus, mitoxantrone has the potential to be carcinogenic in humans.

Reproductive Toxicity

Mitoxantrone was tested for reproductive toxicity in a combination Segment I study and two Segment II studies (in rats and rabbits). In all these studies, the dose mitoxantrone was high enough to cause slight toxicity

RECOMMENDATIONS:

Mitoxantrone is a drug with a narrow therapeutic index. The lethal doses in experimental animals are within a factor of two of the proposed clinical dose. In addition, mitoxantrone is carcinogenic in two species (mouse and rat) at doses well below the proposed clinical doses in humans. These carcinogenic effects occurred at doses which had no effects on white blood cell counts or caused any other sign of toxicity. Since mitoxantrone is genotoxic in a variety of in vivo and in vitro assays, it is likely that mitoxantrone is carcinogenic due to its interaction with DNA rather than by any secondary mechanism, such as immunosuppression. The potential for carcinogenicity and myelosuppression are addressed in the labeling, which serves to alert physicians to these serious toxicities.

Pending labeling revision (see labeling recommendations made in the Summary), the NDA is approvable with respect to the pharmacology/toxicology portion.

/S/

Paul L. Roney, Ph.D.

cc: NDA21120
HFD-120
/G. Fitzgerald
/P. Roney
/T. Wheelous

/S/ 11/15/99

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Appendix I: Statistical Review and Evaluation of Mitoxantrone Carcinogenicity Studies by K. Lin, Ph.D.

Statistical Review and Evaluation

Date: January 27, 1988

NDA #: 19 - 297

Applicant: Lederle Laboratories

Name of Drug: Novantrone Mitoxantrone Hydrochloride (CL 232,315)

Documents Reviewed:

Volumes 1 - 9, Submission of NDA 19-297, Novantrone Mitoxantrone Hydrochloride, Preclinical Studies, dated March 19, 1987.

I. Background

Results of three animal (two mouse and one rat) carcinogenicity studies were submitted in this NDA. These three studies were intended to assess the oncogenic potential of Novantrone Mitoxantrone Hydrochloride in rats and mice when administered intravenously to these animals once every 21 days for two years. Dr. David J. Richman, HFN-150, who is the reviewing pharmacologist on this NDA requested the Division of Biometrics to perform the statistical review and evaluation of the three studies. In order to verify the results submitted and to perform additional analyses, the Division of Biometrics requested that the sponsor provided FDA with the tumor data sets of the three studies. Data tapes NB2533, NB2790 and NA3380 containing SAS data sets, and SAS Proc CONTENTS of the first mouse, the second mouse, and the rat studies, respectively, were received from the sponsor January 12, 1987, and October 21, 1987. These data sets were used in the reviewer's analysis.

II. The First Mouse Study

II.a Design

In this first mouse study, 300 male and 300 female mice were randomly and equally distributed among 5 groups of 60 males and 60 females each. Three groups received mitoxantrone intravenously at doses of 0.1, 0.2, or 0.4 mg/kg once every 21 days for two years. The other groups served as

controls, one was untreated and the other received saline vehicle intravenously also once every 21 days for two years. Animals survived at the end of the study periods were sacrificed for postmortem examinations.

II.b Sponsor's Analyses

The method given in the paper of Kaplan and Meier (Journal of American Statistical Association, 53, pp 457-481, 1958) was applied to the intercurrent mortality data of each sex/treatment group to calculate survival probabilities and survival curves in the study. For each sex, the Cox logrank procedure given in the paper of Cox (Journal of Royal Statistical Society. Series B, 34, pp 187-220, 1972) and the Tarone procedure given in the paper of Tarone (Biometrika, 62, pp 679-682, 1975) were used to test significant dose-response relationships in intercurrent mortality in both male and female mice. The sponsor analyses showed that survivals of the low and medium dose groups were similar to those of the two control groups combined for both sexes. However, there is very significant positive dose-response relationship in intercurrent mortality in both male and female mice (both with $p < 0.0001$). The mortality increased very significantly after approximately 300 days after treatment in high dose male mice, and approximately 440 days after treatments in high dose female mice.

The method given in the paper of Peto et. al. (IARC monographs, Supplement 2, pp 311-426, 1980, WHO) and the procedure given in the paper of Tarone (1975) were used to analyze the incidental tumor rate data. The sponsor's analyses on the data of incidental tumors with more than five occurrences showed that the positive dose-response relationship is significant in liver hepatocellular adenoma ($p = 0.0053$) and in hepatocellular carcinoma ($p = 0.0049$) in male mice. When the incidence rates of the two tumors were combined, the positive dose-response relationship is significant ($p = 0.0001$).

II.c Reviewer's Analyses

The data of the two control groups were combined in our analyses. The numbers of animals dying and the numbers of animals at risk at the beginning of each time interval for both male and female mice are given in Table 1. The survival rates at the times of terminal sacrifice for the control, low, medium, and high dose groups were 28%, 28%, 15%, and 3%, respectively, in males, and 23%, 23%, 20%, and 2%, respectively, in females. The intercurrent mortality rates were tested for dose-response relationship according to the method given in the paper of Peto, et. al. (1980). The results of the analyses (in Table 2) show that there is a significant positive dose-response relationship in intercurrent mortality rate in both males ($p < 0.00001$) and females ($p < 0.00001$).

The Peto prevalence analysis of incidental tumors given in the paper Peto et. al. (1980) using time intervals (in weeks) 0 - 50, 51 - 80, 81 - 104, and terminal sacrifices was used to test the positive dose-response relationship in 17 tumor/sex combinations. The 17 selected tumor/sex combinations are those tumor types with five or more occurrences across treatment groups within a sex group. The incidence rates of these tumors and the results of the individual analyses are given in Tables M1.1 - M1.17. Table 3 contains the p-values of the 17 tests. Among the 17 tumor/sex combinations tested, only liver adenoma hepatocellular in male mice shows significant positive dose-response relationship among the treatment groups ($p = 0.0085$).

III. The Second Mouse Study

III.a Design

In this second mouse study, 240 male and 240 female mice were randomly and equally divided into four groups of 60 males and 60 females each. Three groups received mitoxantrone intravenously at doses of 0.01, 0.03, or 0.06 mg/kg once every 21 days for two years. The fourth group served as control and received saline vehicle intravenously also once every 21 days for two years. Animals survived at the end of the study periods were sacrificed for postmortem examinations.

III.b Sponsor's Analyses

The sponsor showed that the mortality rates after 25 months were 60%, 52%, 47%, and 65% in males and 58%, 68%, 60%, and 65%, in females, in the control, low, medium, and high dose groups, respectively. Their tests showed that there are no significant dose-response relationship in intercurrent mortality for both sexes ($p = 0.3615$ in male mice, $p = 0.3615$ in female mice).

The sponsor's analyses on data of incidental tumors with more than 5 occurrences showed that there is no significant positive dose-response relationship in all the tumors tested (separately or combined) in either male or female mice.

III.c Reviewer's Analyses

The numbers of animals dying and the numbers of animal at risk at the beginning of each time interval for both male and female mice in this study are presented in Table 4. The survival rates at the times of terminal sacrifices were 42%, 50%, 55%, and 35%, respectively, in males, and 43%, 33%, 42%, and 40%, respectively, in females. These rates were tested for

dose-response relationship according to the method given in Peto et. al. (1970). The results of the analyses (in Table 5) show that there is no significant dose-response relationship (positive or negative) in mortality rate in male and female mice ($p = 0.5553$ and $p = 0.9502$, respectively).

Eight tumor/sex combinations with five or more occurrences across treatment groups within a sex group were tested in this study for positive dose-response relationship according to the method of analyzing incidental tumors given in Peto et. al. (1980). The tumor incidence rates and the analysis results of the individual tumor/sex combinations are given in Table M2.1 - M2.8. These analysis results are summarized in Table 6. The results of the tests show that there is no significant positive dose-response relationship in the eight tumor/sex combinations tested.

IV. The Rat Study

IV.a Design

In this rat study, 320 male and 320 female rats were randomly and equally divided into five groups. Three groups of 60 males and 60 females each received mitoxantrone intravenously at doses of 0.01, 0.03, and 0.10 mg/kg once every 21 days for approximately 25 months. The other two groups of 70 males and 70 females each served as two controls and received saline vehicle intravenously also once every 21 days for about 25 months. Animals survived at the end of the study periods were sacrificed for postmortem examinations.

IV.b Sponsor's Analyses

The sponsor's analysis results showed very significant positive dose-response relationship in intercurrent mortality in both male and female rats (both with $p < 0.0001$) . The mortality of the high dose male rats increased very significantly approximately 400 days after the treatment. The mortality rate of the high dose female rats also increased significantly about 500 days after treatment although the magnitudes of increases were not as large as those shown in the high dose male rats.

Finally, the sponsor analyses on the data of incidental tumors with more than five occurrences showed significant positive dose-response relationships in the following tumors: in skin external auditory canal tumor in both male rats ($p < 0.0001$) and in female rats ($p < 0.0001$).

IV.c Reviewer's Analysis

The data of the two control groups in this study were combined in the reviewer's analyses. The numbers of animals dying and the numbers of

animals at risk at the beginning of each time interval for both male and female rats are given in Table 7. The survival rates at the times of terminal sacrifices for the control, low, medium, and high dose groups were 29%, 37%, 18%, and 0%, respectively, in male rats, and 54%, 30%, 33%, and 15%, respectively, in female rats. The intercurrent mortality rates were tested for dose-response relationship according to the method given in Peto et. al. (1980). The results of the tests are given in Table 8. Like the first mouse study, there is a significant positive dose-response relationship in both male and female rats (both with $p < 0.00001$). The high dose male rats show much higher mortality rates than those of the male rats in other groups during the second year of the study. There is no male rat in the high dose group survived at the end of week 106 for terminal sacrifice as shown in Table 7.

The Peto prevalence analysis of incidental tumors given in the paper Peto et. al. (1980) was used to test the positive dose-response relationship in 11 tumor/sex combinations. The 11 selected tumor/sex combinations are those tumor types with five or more occurrences across treatment groups within a sex group. The tumor incidence rates and the analysis results of individual tumor/sex combinations are presented in Tables R.1 - R.11. The results of the tests (the p-values) are given in Table 9. The test results show a significant positive dose-response relationship in skin external auditory canal in both male rats ($p = 0.00071$) and female rats ($p = 0.000005$), and in skin fibroma in female rats ($p = 0.00006$). The results also show a significant negative dose-response relationship in pituitary gland adenoma in male rats. The excessive mortality rates of male rats in the high dose group could have different effects on the above significant positive and negative dose-response relationships in male rats. If the mortality rates of male rats in the high dose group are lower so that there were animals survived at the end of week 106, some of those male rats may be found to have skin external auditory canal tumor, or pituitary gland adenoma during terminal sacrifices. This will make the significant positive dose-response relationship in skin external auditory canal tumor more significant, and the significant negative dose-response relationship in pituitary gland adenoma less significant in male rats.

V. Summary

V.a The First Mouse Study

In this first animal carcinogenicity study, novantrone was evaluated for its oncogenic potential in mice when administered intravenously to both sexes at dose levels of 0.1, 0.2, or 0.4 mg/kg once every 21 days for two years. The statistical methods given in the paper of Peto et. al. (1980) were used to test positive dose-response relationships in intercurrent mortality rate and tumor incidence rate.

Our analyses show that there is a significant positive dose-response relationship in intercurrent rate in both male and female mice (both with $p < 0.00001$). Among the incidental tumor types with more than 5 occurrences, only liver adenoma hepatocellular in male mice shows significant positive dose-response relationship.

V.b The Second Mouse Study

Novantrone was evaluated for its oncogenic potential in mice in this second two-year study when administered intravenously to both sexes at doses of 0.01, 0.03, or 0.06 mg/kg once every 21 days. The statistical methods given in the paper of Peto et. al. (1980) were used to test positive dose-response relationships in intercurrent mortality rate and tumor incidence rate.

Our analyses show that there is no significant dose-response relationship in intercurrent mortality rate in both male and female mice. Nor is there significant positive dose-response relationship in any of the incidental tumors with more than five occurrences in both sexes.

V.c The Rat Study

The oncogenic potential of novantrone was evaluated in this rat study when administered intravenously to both male and female rats at dose levels of 0.01, 0.03, and 0.10 mg/kg once every 21 days. The statistical methods given in the paper of Peto et. al. (1980) were used to test positive dose-response relationship in intercurrent mortality and incidental tumor rates.

Our analyses show that there is a significant dose-response relationship in mortality rate in both male and female rats; and that there is a positive dose-response relationship in skin external auditory canal in both male and female rats, and in skin fibroma in female rats.

VI. Discussions

The results of our analyses agree in general with those submitted by the sponsor. Besides the significant positive dose-relationships in skin external auditory canal in male and female rats, and skin fibroma in female rats, novantrone did not show significant carcinogenic effects on the two species of animals. However, before drawing the final conclusion of little oncogenic potential, it is important to examine the validity of the designs used. The main things in determining the validity of an experiment are: (1). The numbers of animals alive over the course of the study to get an

adequate exposure to the chemical and to be at risk of forming late-developing tumors. (2). If the doses are toxic enough to present a reasonable tumor challenge to the animals. The sponsor did not address those issues.

There is no agreement among experts on those issues. The adequate numbers of animals alive, length of exposure, and dose strength depend on species and strain of animals employed, route of administration, and other factors. The following criteria or rules of thumb have been proposed by some experts in the field:

A. Adequacy of animal exposure to the chemical.

A.1. Proposed by Haseman (through personal communication)

A 50% survival rate of the 50 initial animals in the high dose group between weeks 80 - 90 will be considered as a sufficient number and an adequate exposure. However, the percentage can be higher or lower if the number of animals used in each treatment/sex is larger or smaller than 50 so there will be between 20 to 30 animals still alive during these weeks.

A.2. Proposed by Chu, Cueto, and Ward (Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays, Journal of Toxicology and Environmental Health, 8, 1981, pp 251-280)

To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have (high dose) groups of animals with greater than 50% survival at one year (52 weeks).

B. If high dose animals received maximum tolerated dose (MTD) to get a reasonable tumor challenge.

Criteria proposed by Chu et. al. (1981).

B.1. If there is a detectable weight loss up to 10% in the dosed groups relative to the control.

B.2. If the dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical.

B.3. If the dosed animals show a slightly increased mortality compared to the controls.

In this submission, the survival rates of the high dose group in the first mouse study at the beginning of week 51 and week 81 are 70% and 18% in male mice, and 97% and 25% in female mice. The survival rates in the second mouse study at the beginning of the two weeks are 98% and 78% in male mice, and 97% and 78% in female mice. In the rat study, the survival rates are 100% and 33% in the male rats, and 98% and 80% in female rats.

In terms of the criterion A.2., the animals used in the three studies got an adequate exposure to the chemical. However, both high dose males and females in the first mouse study, and high dose males in the rat study show excessive mortality rates in terms of criterion A.1.

Both our and the sponsor's analyses show that there is no activity of any tumor type in the second mouse study. In this study, there were sufficient animals in the high dose group remain alive long enough to get an adequate exposure to the chemical. However, because of the dose level (0.06 mg/kg) applied to the high dose group animals, it showed (in terms of criteria B.1. and B.3.) that the high dose level was not high enough to present a reasonable challenge to the animals.

/S/

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Mathematical Statistician

Concur: Suresh C. Rastogi, Ph.D. /S/ 1/27/

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cc: Original NDA 19-297 /S/
HFN-150/Dr. Palme
HFN-150/Dr. Richman
HFN-710/Chron
HFN-715/Dr. Fairweather
HFN-715/Dr. Rastogi
HFN-715/Dr. Lin
HFN-715/DRU 2.1.1, Novantrone, Lederle Laboratories
HFN-715/KKLin/01/14/88/443-4710/nmNDA19297/

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Table 1

Intercurrent Mortality Rates

The First Two-year Mouse Study

Male Mice

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	120	15	13	60	9	15	60	5	8	60	17	28
51 - 80	105	26	25	51	13	25	55	20	36	43	32	74
81 - 104	79	45	57	38	21	55	35	26	74	11	9	82
Terminal	34			17			9			2		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the period.

Female Mice

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	120	8	7	60	5	8	60	5	8	60	2	3
51 - 80	112	17	15	55	11	20	55	13	24	58	43	74
81 - 104	95	67	71	44	30	68	42	30	71	15	14	93
Terminal	28			14			12			1		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the period.

Table 2

Peto Intercurrent Mortality Analysis

The First of the Two Mouse Studies

ANIMAL	SEX	TOTAL	VALUE	VARIANCE	Z-VALUE	P-VALUE *
Mouse	Male	Trend	9.91128	2.58455	6.16596	< 0.00001
Mouse	Female	Trend	9.56551	2.31982	6.28031	< 0.00001

Notes: The Time Interval Used in the Above Analysis are (in Week) 0 - 50, 51 - 80, and 81 - 104.
* Two-tailed.

Table 3

Results of Peto Prevalence Analyses of Incidental Tumors

The First of the Two Mouse Studies

ORGAN	TUMOR	SEX	P-VALUE #
Adrenals	Adenoma-Cortical	M	0.7044
Liver	Adenoma Hepatocellular	M	0.0085 *
	Carcinoma Hepatocellular	M	0.0385
	Carcinoma Hepatocellular	F	0.7118
Lung	Adenocarcinoma-Alveolar/Bronchiolar	M	0.4120
		F	0.3299
	Adenoma, Alveolar Cell	M	0.0910
	Alveolar Cell Carcinoma	M	0.6929
		F	0.8731
	Mammary Adenocarcinoma, Metastatic	F	0.4735
Mammary Gland	Adenocarcinoma-Lobular	F	0.5677
	Fibroadenoma	F	0.1214
Pituitary Gland	Adenoma	F	0.7930
Uterus	Cystadenoma, Papillary	F	0.3781
	Endometrial Polyp	F	0.2705
	Leiomyoma	F	0.6793
	Leiomyosarcoma	F	0.8198

Notes: # : One-sided P-value.
* : Significant at level 0.01.

APPEARS THIS WAY
ON ORIGINAL

Table 4

Intercurrent Mortality Rates

The Second Two-Year Mouse Study

Male Mice

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	60	5	8	60	4	7	60	5	8	60	1	2
51 - 80	55	11	20	56	8	14	55	9	16	59	12	20
81 - 105	44	19	43	48	18	38	46	13	28	47	26	55
Terminal	25			30			33			21		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the Period.

Female Mice

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	60	3	5	60	2	3	60	6	10	60	2	3
51 - 80	57	8	14	58	11	19	54	9	17	58	11	19
81 - 105	49	23	47	47	27	57	45	20	44	47	23	49
Terminal	26			20			25			24		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the Period.

Table 5

Peto Intercurrent Mortality Analysis

The Second of the Two Mouse Studies

ANIMAL	SEX	TOTAL	VALUE	VARIANCE	Z-VALUE	P-VALUE *
Mouse	Male	Trend	0.13018	0.048722	0.589781	0.555338
Mouse	Female	Trend	0.01382	0.048887	0.062513	0.950154

Notes: The Time Interval Used in the Above Analysis are (in Week) 0 - 50, 51 - 80, and 81 - 104.
* Two-tailed.

APPEARS THIS WAY
ON ORIGINAL

Table 6

Results of Peto Prevalence Analyses of Incidental Tumors

The Second of the Two Mouse Studies

ORGAN	TUMOR	SEX	P-VALUE #
Liver	Adenoma, Hepatocellular	M	0.2993
	Carcinoma Hepatocellular	M	0.9091
Lung	Adenoma, Alveolar Cell	M	0.2989
		F	0.2796
	Alveolar Cell Carcinoma	M	0.8271
		F	0.3368
Mammary Gland	Adenocarcinoma-Lobular	F	0.9632
Pituitary Gland	Adenoma	F	0.6579

Notes: One-sided P-value.

APPEARS THIS WAY
ON ORIGINAL

Table 7

Intercurrent Mortality Rates

The Two-year Rat Study

Male Rats

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	140	6	4	60	3	5	60	0	0	60	0	0
51 - 80	134	24	18	57	6	11	60	13	22	60	40	67
81 - 106	110	69	63	51	29	57	47	36	77	20	20	100
Terminal	41			22			11			0		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the Period.

Female Rats

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	ST	D	%	ST	D	%	ST	D	%	ST	D	%
0 - 50	140	3	2	60	0	0	60	3	5	60	1	2
51 - 80	137	7	5	60	10	17	57	9	16	59	11	19
81 - 110	130	55	42	50	32	64	48	28	58	48	39	81
Terminal	75			18			20			9		

Notes: ST = Number of Animals at the Beginning of Each Interval.
D = Number of Death During the Period.
% = Percent of Death During the Period.

APPEARS THIS WAY
ON ORIGINAL

Table 8

Peto Intercurrent Mortality Analysis

The Rat Study

ANIMAL	SEX	TOTAL	VALUE	VARIANCE	Z-VALUE	P-VALUE *
Mouse	Male	Trend	2.73224	0.13696	7.38282	< 0.00001
Mouse	Female	Trend	1.80501	0.14461	4.74661	< 0.00001

Notes: The Time Interval Used in the Above Analysis are (in Week) 0 - 50, 51 - 80, and 81 - 104.
* Two-tailed.

APPEARS THIS WAY
ON ORIGINAL

Table 9

Results of Peto Prevalence Analyses of Incidental Tumors

The Rat Study

ORGAN	TUMOR	SEX	P-VALUE #
Skin	External Auditory Canal	M	0.00071 *
		F	0.000005 *
	Fibroma	M	0.3374
		F	0.00006 *
Mammary Gland	*Fibroadenoma*	*F*	*0.1699*
Pancreas	Islet Cell Tumor	M	0.9677
		F	0.2443
Adrenal Glands	Medullary Tumor	M	0.1412
		F	0.3067
Pituitary Gland	Adenoma	M	0.99996
		F	0.9639

Notes: # : One-sided P-value.
* : Significant at level 0.01.

Table M1.1

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Adrenals, Adenoma-Cortical

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	0	17	0
51 - 80	0	26	0	0	13	0	0	20	0	0	32	0
81 - 104	4	45	9	2	21	10	2	26	8	0	9	0
Term Sacri	1	34	3	4	17	24	0	9	0	0	2	0
Total	5			6			2			0		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.2101	0.15308	-0.53709	0.7044

APPEARS THIS WAY
ON ORIGINAL

Table M1.2

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Liver, Adenoma-Hepatocellular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	0	17	0
51 - 80	0	26	0	0	13	0	0	20	0	1	32	3
81 - 104	1	45	2	2	21	10	3	26	12	2	9	22
Term Sacri	1	34	3	6	17	35	0	9	0	0	2	0
Total	2			8			3			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	1.05225	0.19459	2.38537	0.0085

APPEARS THIS WAY
ON ORIGINAL

Table M1.3

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Liver, Carcinoma-Hepatocellular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	0	17	0
51 - 80	2	26	8	0	13	0	2	20	10	4	32	13
81 - 104	9	45	20	3	21	14	5	26	19	2	9	22
Term Sacri	4	34	12	4	17	24	5	9	56	1	2	50
Total	15			7			12			7		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	1.28733	0.530159	1.76802	0.0385

APPEARS THIS WAY
ON ORIGINAL

Table M1.4

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Liver, Carcinoma-Hepatocellular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	0	13	0	0	43	0
81 - 104	3	67	4	1	30	3	0	30	0	1	14	7
Term Sacri	2	28	7	0	14	0	0	12	0	0	1	0
Total	5			1			0			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.170458	0.093149	-0.558505	0.71175

APPEARS THIS WAY
ON ORIGINAL

Table M1.5

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Lungs, Adenocarcinoma-Alveolar/Bronchiolar

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	0	17	0
51 - 80	1	26	4	0	13	0	1	20	5	0	32	0
81 - 104	2	45	4	2	21	10	2	26	8	0	9	0
Term Sacri	3	34	9	3	17	18	1	9	11	1	2	50
Total	6			5			4			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.099834	0.20170	0.222295	0.4120

APPEARS THIS WAY
ON ORIGINAL

Table M1.6

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Lungs, Adenocarcinoma-Alveolar/Bronchiolar

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	0	13	0	1	43	2
81 - 104	4	67	6	3	30	10	4	30	13	C	14	0
Term Sacri	1	28	4	2	14	14	1	12	8	0	1	0
Total	5			5			5			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.206729	0.22046	0.44029	0.3299

APPEARS THIS WAY
ON ORIGINAL

Table M1.7

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Lungs, Adenoma, Alveolar Cell

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	0	17	0
51 - 80	0	26	0	0	13	0	0	20	0	0	32	0
81 - 104	1	45	2	0	21	0	2	26	8	1	9	11
Term Sacri	3	34	9	0	17	0	2	9	22	0	2	0
Total	4			0			4			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.42154	0.099728	1.33485	0.09096

APPEARS THIS WAY
ON ORIGINAL

Table M1.8

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Male Mice, Lungs, Alveolar Cell, Carcinoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	15	0	0	9	0	0	5	0	1	17	6
51 - 80	1	26	4	1	13	8	0	20	0	1	32	3
81 - 104	6	45	13	1	21	5	2	26	8	0	9	0
Term Sacri	1	34	3	0	17	0	3	9	33	0	2	0
Total	8			2			5			2		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.24541	0.236909	-0.504198	0.6929

APPEARS THIS WAY
ON ORIGINAL

Table M1.9

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Lungs, Alveolar Cell, Carcinoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	1	2	50
51 - 80	1	17	6	1	11	9	0	13	0	0	43	0
81 - 104	2	67	3	2	30	7	2	30	7	1	14	7
Term Sacri	7	28	25	0	14	0	1	12	8	0	1	0
Total	10			2			3			2		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.533351	0.21836	-1.14137	0.8731

APPEARS THIS WAY
ON ORIGINAL

Table M1.10

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Lungs, Mammary Adenocarcinoma, Metastatic

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	1	11	9	0	13	0	2	43	5
81 - 104	4	67	6	2	30	7	0	30	0	1	14	7
Term Sacri	0	28	0	0	14	0	0	12	0	0	1	0
Total	4			3			0			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.028749	0.18639	0.06659	0.4735

APPEARS THIS WAY
ON ORIGINAL

Table M1.11

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Mammary Gland, Adenocarcinoma-Lobular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	1	17	6	1	11	9	0	13	0	3	43	7
81 - 104	9	67	13	4	30	13	1	30	3	2	14	14
Term Sacri	1	28	4	2	14	14	1	12	8	0	1	0
Total	11			7			2			5		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.10624	0.38817	-0.17052	0.5677

APPEARS THIS WAY
ON ORIGINAL

Table M1.12

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Mammary Gland, Fibroadenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	0	13	0	0	43	0
81 - 104	0	67	0	2	30	7	1	30	3	1	14	7
Term Sacri	1	28	4	0	14	0	0	12	0	0	1	0
Total	1			2			1			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.309452	0.07021	1.16788	0.1214

APPEARS THIS WAY
ON ORIGINAL

Table M1.13

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Pituitary Gland, Adenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	2	17	12	0	11	0	1	13	8	0	43	0
81 - 104	7	67	10	2	30	7	3	30	10	1	14	7
Term Sacri	6	28	21	2	14	14	5	12	42	0	1	0
Total	15			4			9			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.485255	0.35277	-0.81700	0.7930

APPEARS THIS WAY
ON ORIGINAL

Table M1.13

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Pituitary Gland, Adenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	2	17	12	0	11	0	1	13	8	0	43	0
81 - 104	7	67	10	2	30	7	3	30	10	1	14	7
Term Sacri	6	28	21	2	14	14	5	12	42	0	1	0
Total	15			4			9			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.485255	0.35277	-0.81700	0.7930

APPEARS THIS WAY
ON ORIGINAL

Table M.14

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Uterus, Cystadenoma, Papillary

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	2	13	15	2	43	5
81 - 104	5	67	7	0	30	0	1	30	3	1	14	7
Term Sacri	1	28	4	1	14	7	1	12	8	0	1	0
Total	6			1			4			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.150848	0.236022	0.310502	0.3781

APPEARS THIS WAY
ON ORIGINAL

Table M1.15

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study -

Female Mice, Uterus, Endometrial Polyp

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	1	8	13	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	0	13	0	2	43	5
81 - 104	6	67	9	2	30	7	2	30	7	0	14	0
Term Sacri	2	28	7	1	14	7	3	12	25	1	1	100
Total	9			3			5			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.317375	0.269439	0.611423	0.2705

APPEARS THIS WAY
ON ORIGINAL

Table M1.16

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Uterus, Leiomyoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	0	17	0	0	11	0	0	13	0	1	43	2
81 - 104	3	67	4	4	30	13	2	30	7	0	14	0
Term Sacri	4	28	14	3	14	21	0	12	0	0	1	0
Total	7			7			2			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.21527	0.213557	-0.465829	0.6793

APPEARS THIS WAY
ON ORIGINAL

Table M1.17

Peto Prevalence Analysis of Incidental Tumors

The First Two-year Mouse Study

Female Mice, Uterus, Leiomyosarcoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	8	0	0	5	0	0	5	0	0	2	0
51 - 80	1	17	6	0	11	0	1	13	8	0	43	0
81 - 104	2	67	3	0	30	0	2	30	7	0	14	0
Term Sacri	0	28	0	0	14	0	0	12	0	0	1	0
Total	3			0			3			0		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.311803	0.116192	-0.914729	0.8198

APPEARS THIS WAY
ON ORIGINAL

Table M2.1

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Male Mice, Liver, Adenoma, Hepatocellular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	5	0	0	4	0	0	5	0	0	1	0
51 - 80	0	11	0	2	8	25	0	9	0	0	12	0
81 - 105	1	19	5	0	18	0	1	13	8	2	26	8
Term Sacri	3	25	12	3	30	10	1	33	3	4	21	19
Total	4			5			2			6		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.047055	0.007993	0.526311	0.2993

APPEARS THIS WAY
ON ORIGINAL

Table M2.2

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Male Mice, Liver, Carcinoma, Hepatocellular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	5	0	0	4	0	0	5	0	0	1	0
51 - 80	3	11	28	0	8	0	0	9	0	1	12	8
81 - 105	2	19	11	2	18	11	1	13	8	3	26	12
Term Sacri	6	25	24	2	30	7	4	33	12	1	21	5
Total	11			4			5			5		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.145339	0.011848	-1.33522	0.9091

APPEARS THIS WAY
ON ORIGINAL

Table M2.3

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Male Mice, Lungs, Adenoma, Alveolar Cell

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	5	0	0	4	0	0	5	0	0	1	0
51 - 80	1	11	9	1	8	13	1	9	11	0	12	0
81 - 105	1	19	5	1	18	6	0	13	0	2	26	8
Term Sacri	3	25	12	3	30	10	6	33	18	4	21	19
Total	5			5			7			6		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.053333	0.010214	0.527703	0.2989

APPEARS THIS WAY
ON ORIGINAL

Table M2.4

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Female Mice, Lungs, Adenoma, Alveolar Cell

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	2	0	0	6	0	0	2	0
51 - 80	0	8	0	0	11	0	0	9	0	0	11	0
81 - 105	0	23	0	4	27	15	3	20	15	4	23	17
Term Sacri	2	26	8	1	20	5	3	25	12	0	24	0
Total	2			5			6			4		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.052924	0.0082087	0.584135	0.2796

APPEARS THIS WAY
ON ORIGINAL

Table M2.5

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Male Mice, Lungs, Alveolar Cell Carcinoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	5	0	0	4	0	0	5	0	0	1	0
51 - 80	0	11	0	0	8	0	0	9	0	0	12	0
81 - 105	1	19	5	2	18	11	2	13	15	1	26	4
Term Sacri	3	25	12	3	30	10	3	33	9	1	21	5
Total	4			5			5			2		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.082103	0.0075838	-0.942791	0.8271

APPEARS THIS WAY
ON ORIGINAL

Table M2.6

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Female Mice, Lungs, Alveolar Cell Carcinoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	2	0	0	6	0	0	2	0
51 - 80	0	8	0	1	11	9	0	9	0	2	11	18
81 - 105	1	23	4	2	27	7	0	20	0	0	23	0
Term Sacri	1	26	4	1	20	5	1	25	4	2	24	8
Total	2			4			1			4		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.031630	0.005642	0.421093	0.3368

APPEARS THIS WAY
ON ORIGINAL

Table M..7

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Female Mice, Mammary Gland, Adenocarcinoma-Lobular

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	2	0	0	6	0	0	2	0
51 - 80	1	8	13	0	11	0	1	9	11	0	11	0
81 - 105	2	23	9	1	27	4	1	20	5	1	23	4
Term Sacri	4	26	15	0	20	0	1	25	4	0	24	0
Total	7			1			3			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.140091	0.006134	-1.78875	0.9632

APPEARS THIS WAY
ON ORIGINAL

Table M2.8

Peto Prevalence Analysis of Incidental Tumors

The Second Two-year Mouse Study

Female Mice, Pituitary Gland, Adenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	2	0	0	6	0	0	2	0
51 - 80	0	8	0	1	11	9	0	9	0	0	11	0
81 - 105	0	23	0	0	27	0	0	20	0	2	23	9
Term Sacri	4	26	15	2	20	10	2	25	8	1	24	4
Total	4			3			2			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.031475	0.0059845	-0.406863	0.6579

APPEARS THIS WAY
ON ORIGINAL

Table R.1

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Male Rats, Skin, External Auditory Canal

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	6	0	0	3	0	0	0	-	0	0	-
51 - 80	2	24	8	0	6	0	0	13	0	4	40	10
81 - 106	0	69	0	0	29	0	3	36	8	4	20	20
Term Sacri	0	41	0	1	22	5	1	11	9	0	0	-
Total	2			1			4			8		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.440267	0.019061	3.18891	0.00071

APPEARS THIS WAY
ON ORIGINAL

Table R.2

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Skin, External Auditory Canal

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	0	-	0	3	0	0	1	0
51 - 80	0	7	0	0	10	0	0	9	0	2	11	18
81 - 110	1	55	2	0	32	0	0	28	0	5	39	13
Term Sacri	0	75	0	0	18	0	1	20	5	1	9	11
Total	1			0			1			8		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.525857	0.014102	4.42827	0.000005

APPEARS THIS WAY
ON ORIGINAL

Table R.3

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Male Rats, Skin, Fibroma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	6	0	0	3	0	0	0	-	0	0	-
51 - 80	1	24	4	0	6	0	0	13	0	3	40	8
81 - 106	4	69	6	1	29	3	1	36	3	1	20	5
Term Sacri	6	41	15	1	22	5	1	11	9	0	0	-
Total	11			2			2			4		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTALS	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.05290	0.015904	0.41948	0.3374

APPEARS THIS WAY
ON ORIGINAL

Table E.4

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Skin, Fibroma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	0	-	0	3	0	0	1	0
51 - 80	0	7	0	0	10	0	0	9	0	0	11	0
81 - 110	1	55	2	0	32	0	0	28	0	5	39	13
Term Sacri	0	75	0	0	18	0	0	20	0	1	9	11
Total	1			0			0			6		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.38909	0.01021	3.8501	0.00006

APPEARS THIS WAY
ON ORIGINAL

Table R.5

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Mammary Gland, Fibroadenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	0	-	0	3	0	0	1	0
51 - 80	0	7	0	3	10	30	1	9	11	1	11	9
81 - 110	12	55	22	9	32	28	7	28	25	14	39	36
Term Sacri	26	75	35	4	18	22	6	20	30	3	9	33
Total	38			16			14			18		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.264302	0.0766336	0.954753	0.1699

APPEARS THIS WAY
ON ORIGINAL

Table R.6

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Male Rats, Pancreas, Islet Cell Tumor

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	6	0	0	3	0	0	0	-	0	0	-
51 - 80	1	24	4	0	6	0	0	13	0	2	40	5
81 - 106	13	69	19	3	29	10	3	36	8	0	20	0
Term Sacri	12	41	29	3	22	14	2	11	18	0	0	-
Total	26			6			5			2		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.292974	0.02515	-1.8474	0.9677

APPEARS THIS WAY
ON ORIGINAL

Table R.7

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Pancreas, Islet Cell Tumor

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	0	-	0	3	0	0	1	0
51 - 80	0	7	0	0	10	0	0	9	0	0	11	0
81 - 110	2	55	4	4	32	13	4	28	14	1	39	3
Term Sacri	10	75	13	2	18	11	1	20	5	4	9	44
Total	12			6			5			5		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.114473	0.027313	0.692663	0.2443

APPEARS THIS WAY
ON ORIGINAL

Table R.8

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Male Rats, Adrenal Glands, Medullary Tumor

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	6	0	0	3	0	0	0	-	0	0	-
51 - 80	1	24	4	0	6	0	0	13	0	0	40	0
81 - 106	6	69	9	2	29	7	2	36	6	3	20	15
Term Sacri	6	41	15	1	22	5	6	11	55	0	0	-
Total	13			3			8			3		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.135283	0.0158372	1.07499	0.1412

APPEARS THIS WAY
ON ORIGINAL

Table R.9

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Adrenal Glands, Medullary Tumor

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50 [3]	0	3	0	0	0	-	0	3	0	0	1	0
51 - 80	0	7	0	0	10	0	0	9	0	0	11	0
81 - 110	1	55	2	0	32	0	1	28	4	0	39	0
Term Sacri	2	75	3	0	18	0	1	20	5	1	9	11
Total	3			0			2			1		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	0.039204	0.0060242	0.5051	0.3067

APPEARS THIS WAY
ON ORIGINAL

Table R.10

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Male Rats, Pituitary Gland, Adenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	6	0	1	3	33	0	0	-	0	0	-
51 - 80	10	24	42	2	6	33	6	13	46	5	40	13
81 - 106	37	69	54	16	29	55	15	36	42	4	20	20
Term Sacri	25	41	61	9	22	41	7	11	64	0	0	-
Total	72			28			28			9		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

****

Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-1.09678	0.077733	-3.93384	0.99996

APPEARS THIS WAY
ON ORIGINAL

Table R.11

Peto Prevalence Analysis of Incidental Tumors

The Two-year Rat Study

Female Rats, Pituitary Gland, Adenoma

Time Intervals (Weeks)	Groups											
	Control			Low			Medium			High		
	T	N	%	T	N	%	T	N	%	T	N	%
0 - 50	0	3	0	0	0	-	1	3	33	0	1	0
51 - 80	4	7	57	7	10	70	5	9	56	5	11	45
81 - 110	54	55	98	27	32	84	27	28	96	34	39	87
Term Sacri	58	75	77	17	18	94	15	20	75	6	9	67
Total	116			51			48			45		

Notes: T = Number of Necropsies with the Above Tumor.
N = Number of Necropsies.
% = Percent of Necropsies with the Above Tumor.

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Results of the Peto Analysis

Prevalence Analysis of Incidental Tumor

TOTAL	T-VALUE	VARIANCE OF T-VALUE	Z-VALUE	ONE-TAILED P-VALUE
TREND	-0.399149	0.0492772	-1.79809	0.9639

APPEARS THIS WAY
ON ORIGINAL