UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 12 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Chloroneb rat teratology and reproduction studies.
CASWELL #198

TO: Henry Jacoby (21)
Registration Division (TS-767)

FROM: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

D. Stephen Saunders Jr.
10-9-84

THRU: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)
and
William L. Burnam
Chief, Toxicology Branch
HED (TS-769)

LDC 10/10/84

Chemical: Chloroneb; 1,4-dichloro-2,5-dimethoxybenzene; CAS No. 2675-77-6

Action Requested

Review rat teratology study and data addendum for rat reproduction study.

Recommendations

(1) The rat teratology study has been reviewed and is tentatively classified as Core-Supplementary data. A statistically-significant increase in the incidence of delayed fetal ossification was noted at the LDT, 300 mg/kg (gavage). The apparent dose-effect relationship for this phenomenon was inverse, i.e. a significant decrease in ossification delay relative to control was noted at the HDT, 3500 mg/kg, whereas a significant increase was noted at the LDT. This is an unusual finding in the experience of Toxicology Branch. However, because of the linearity of the response and the statistical significance of the effect at the low dose, a NOEL for fetal toxicity cannot be established at this time based on the submitted data (see data review). Additional data is requested from the registrant as to (a) the order of sacrifice of dams, (b) individual animal dose preparation and administration records, (c) historical control data (tabulated by fetal and litter incidence) for at least two years before the present study from the contracting laboratory, and (d) any other data or explanations that the registrant deems relevant. The maternal NOEL will be established after the requested data is submitted.

No compound-related teratogenic effects were noted in this study.

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(2) The teratology range-finding study is classified as Core-Supplementary data. The data presented in this study support the doses of chloroneb used in the main teratology study. Since high dose fetuses were not examined for visceral or skeletal malformations or variations, these data are not useful for assessment of teratogenicity or fetotoxicity.

(3) The rat reproduction study has been classified as Core-Supplementary data. Although no effect on reproduction was noted, because of numerous deficiencies in the methods employed and in the reporting of the study, the data are considered inadequate for establishment of a reproductive NOEL. The only potential treatment-related effect was a decrease in survival to day 21 of high dose pups of the F_{3b} mating. An approximate 25% decrease in relative spleen weight was also noted in high dose female pups, however no correlative necropsy data for these animals was reported, and the age of these animals at necropsy was not clearly specified (see review).

A new study is requested. The registrant is referred to the 1982 Pesticide Assessment Guidelines for information regarding the design and reporting of the repeat reproduction study.

Data Review

Study Title: Embryo-fetal Toxicity Study of Chloroneb in the Rat.

Accession Number: 251459

Study Number: 104-004

Sponsor/Contracting Laboratory: E. I. duPont de Nemours and Co., Inc./ Argus Research Laboratories, Inc.

Report Date/Submitted: 6-27-83/10-3-83

Test Material: Chloroneb; Lot no. C00604-B, B11; 90.0% a.i.

Doses Tested: 0, 300, 1000, 3500 mg/kg/day by gavage.

Methods

A phqtocopy of the submitted methods i: appended. The following points are noted:

1) Dosing in the present study was conducted on days 5-14 of gestation, in contrast to the minimum data requirement which states that rats should be dosed on days 6-15. The registrant submitted several justifications for this deviation from Agency guidelines, the only relevant one being that "the interval is that during which organogenesis occurs, rather than including the interval of fetal growth". This deviation does not compromise the scientific validity of this study in the opinion of this reviewer.

2) Several references are made in the methods section of the submitted study to "Protocol Addendum 1". No such document is listed in the table of contents, nor could it be located in the submitted study.

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Results

A. Maternal Data- (1) Clinical Signs and Mortality- No dams died during this study as a result of treatment with the test compound. Several physical signs were observed in rats that received 3500 mg/kg chloroneb, however only salivation was noted in rats from the 1000 mg/kg group. No significant physical signs were reported for rats dosed with 300 mg/kg. Hair loss (alopecia) was noted in all treatment groups, however the decreased incidence of this sign in the low dose group and increase in the high dose group are of doubtful toxicological significance. These data are presented in table 1.

Table 1. The Effect of Treatment on Maternal Physical Signs[a]

	Dose (mg/kg)			
Physical Signs	0	300	1000	3500
-salivation	0/25[b]	0/25	24/25*** (84)[c]	25/25*** (198)***
-urine-stained abdominal fur	0/25	0/25	0/25	17/25*** (65)***
-red exudate on abdomen	0/25	0/25	0/25	4/25 (12)***

[a]data and statistics excerpted from tables 1 and 2 of submitted study.

[b]incidence/total number of animals in group.

[c]number of days on which sign was observed.

***$p \leq 0.001$ by Fisher's exact test.

(2) Maternal Weight Gain and Food Consumption- Body weight gain was significantly decreased only in dams treated with the mid and high doses of the test article if calculated over days 5-20 or 0-20 (table 2). However, it can be seen from table 2 that the principal effect on body weight gain occurred over days 5-8, when a statistically significant, dose-related decrease in body weight gain was noted in all treatment groups. Body weight gain over the remainder of the treatment period (i.e. days 9-14) was similar in all treatment groups. From days 14-20, the high dose (3500 mg/kg) group was the only treatment group different from control, with a decrease in average weight gain of about 5% less than control animals. The apparently dose-related decreases in weight gain noted for days 5-14, 5-20, and 0-20 in the low, mid and high dose groups are therefore due to the profound effect of the test article on weight gain over days 5-8, and are not the result of cumulative toxicity.

Table 2. Effect of Treatment on Maternal Body Weight Gain[a]

Days	Dose (mg/kg) 0	300	1000	3500
5-8	12.6± 3.8	8.2± 8.1††	6.3± 7.2†††	0.2±13.0†††
8-12	19.6± 4.5	18.6± 5.0	18.8± 5.5	21.4± 9.1
12-14	10.4± 3.5	11.0± 3.2	9.5± 5.3	10.7± 6.6
14-20	80.5± 9.9	81.0±10.2	79.9± 9.2	75.7± 8.2
5-14	42.6± 7.0	37.8± 7.6†	34.6± 8.6†††	32.3±13.9††
5-20 (with uteri)	123.2±13.5	118.8±14.6	114.5±11.4*	108.0±12.6**
5-20 (minus uteri)	41.7±11.0	38.8±12.0	37.0± 7.4	30.6±11.4**
0-20 (with uteri)	148.4±12.4	143.7±17.0	139.3±12.7*	133.8±12.7**
0-20 (minus uteri)	67.0±10.5	64.1±15.7	61.8±10.5	56.4±11.9**

[a]means ± standard deviation, data and statistics excerpted from submitted study.

†$p \leq 0.05$, ††$p \leq 0.01$, †††$p \leq 0.001$ by Mann-Whitney U Test.

*$p \leq 0.05$, **$p \leq 0.01$ by Dunnett's Least Significant Difference (LSD).

Food consumption paralleled the observed effects of chloroneb on weight gain. Food consumption decreased in all treatment groups on days 5-10 (treatment initiated on day 5), however total food consumption on days 10-15 or 5-15 was significantly decreased only in the groups receiving 1000 and 3500 mg/kg chloroneb (table 3). Food efficiency was calculated by the reviewer to determine if any effect on weight gain other than an effect on food consumption was apparent. Body weights were not determined on the same days as food consumption, so it was necessary to use the day 5-14 body weight values for comparison with the day 5-15 food consumption values. To determine the day 5-20 food efficiency values, a time-weighted average was calculated by the reviewer from food consumption on days 5-15 and 15-20 to estimate the average daily consumption of food over days 5-20. It can be seen from table 3b that food efficiency (i.e. gram of body weight gain/gram food consumed) was approximately equal in all treatment groups at either time interval, but slightly lower than control.

Table 3a. Food Consumption and Efficiency[a]

Days	Dose (mg/kg) 0	300	1000	3500
5-10	22.7±2.0	20.9±3.0*	20.0±2.9**	17.6±2.8**
10-15	24.4±2.0	23.4±2.3	22.9±2.2*	22.1±2.3**
5-15	23.5±1.9	22.2±2.5	21.5±2.2*	19.8±1.6**
15-20	27.4±2.1	27.0±2.4	27.3±2.1	27.6±2.5

Table 3b. Efficiency (%)[b]

5-14	20.1	18.9	17.9	18.1
5-20	33.1	32.2	32.6	32.1

[a]means ± standard deviation, data and statistics excerpted from submitted study.

[b](grams body weight gain/grams food consumed) x 100 = % efficiency

*$p \leq 0.05$, **$p \leq 0.01$ by Dunnett's Least Significant Difference (LSD).

B. Reproductive Data- No significant effects of treatment were observed on the incidence of pregnancy or on the numbers of corpora lutea, implantations, or litters (table 3). Slight increases in the mean number of resorptions and the mean % resorptions per litter were observed in the mid and high dose groups, however these changes were neither statistically significant nor dose-dependent. No animals with total resorption of a litter were noted.

Slight decreases in the number of live fetuses per litter (-7.5% and -4.8%) were observed in dams treated with 1000 and 3500 mg/kg, respectively. These changes approached statistical significance ($p \leq 0.072$ and $p \leq 0.055$, respectively, calculated by the investigators). The investigators stated that the changes may "reflect the slightly lower average numbers of implantation sites for dams of the middle and high dosage groups". This effect may also reflect the slightly higher rates of resorption in these groups.

No stunted (<1.25 grams) or dead fetuses were noted. No effect on fetal body weight was apparent. Reproductive data are presented in table 4.

Table 4. Reproductive and Litter Data[a]

Parameter	Dose (mg/kg) 0	300	1000	3500
No. litters/ mated females	24/25	25/25	25/25	24/25
Total #fetuses	352	373	347	335
#dead fetuses	0	0	0	0
#live/litter	14.7±1.9	14.9±2.2	13.9±2.3	14.0±1.5
Mean birth weight (g)	3.4±0.2	3.3±0.2	3.5±0.3	3.4±0.2
Mean number corpora lutea	17.0±1.8	17.5±1.8	18.0±3.2	16.7±2.4
Mean number implantations	15.4±1.8	15.6±1.9	15.2±1.9	14.9±1.2
Mean number resorptions/litter	0.7±1.0	0.7±0.8	1.4±1.1	0.9±1.0
(mean %)	(4.6±6.3)	(4.6±6.1)	(9.2±8.1)	(6.1±6.8)

[a]data excerpted from submitted study. Values are mean ± std. dev., except where noted (calculated by investigators).

C. Fetal Malformations and Variations- (1) Malformations- No statistically significant or dose-related effects on the total number of animals showing any malformation, % of fetuses with any malformation, % of fetuses/litter with any malformation, or % of litters containing a fetus with a malformation were observed for external, visceral, or skeletal malformations. Malformations related to development of the brain were noted in fetuses from the low and high dose groups. No animals from the mid dose group exhibited any malformations, nor were brain malformations noted in contol fetuses.

One fetus in the control group had unfused eyelids and another had no tail, for a total of 2/352 (0.6%) fetuses and 2/24 (8.3%) litters with an external malformation. On visceral examination, a third fetus was noted to have microphthalmia. A total of 3/24 (12.5%) litters contained a fetus with any malformation.

Four fetuses from three dams treated with 300 mg/kg chloroneb exhibited malformations. On external examination, one had exencephaly, protruding eyes, low-set ears, and protruding tongue, and a littermate had protruding eyes. A fetus from another litter had a shortened body and missing tail, for a total of 3/373 (0.8%) fetuses with an external malformation. On visceral examination, one fetus had hydrocephalus and microphthalmia. A total of 3/25 (12%) of litters from this dose group contained a fetus with any malformation.

No fetuses from dams treated with 1000 mg/kg chloroneb exhibited any malformations (0/177 fetuses, 0/25 litters).

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One fetus (out of 335, 0.3%) from the 3500 mg/kg group was noted to have an external malformation. This fetus had exencephaly, protuding eyes, unfused eyelids, lowset ears, protruding tongue, spina bifida, and anophthalmia. On visceral examination, another fetus was noted to have hydrocephalus. A total of 2/24 litters (8.3%) had a fetus with any malformation.

No fetuses from any group had a skeletal malformation.

Table 5. Fetal Malformations[a]

	Dose (mg/kg)			
External Malformations	0	300	1000	3500
Number examined	352/24[b]	373/25	347/25	335/24
Exencephaly		1/1[c]		1/1[d]
Eyes -protruding		2/1[c]		1/1[d]
-unfused lids	1/1			1/1[d]
Tongue protruding		1/1[c]		1/1[d]
Ears low-set		1/1[c]		1/1[d]
Spina bifida				1/1[d]
Shortened body		1/1[e]		
Missing tail	1/1	1/1[e]		
total	2/2	3/2		1/1
Visceral Malformations				
Number examined	171/24	180/25	170/25	161/24
Hydrocephalus		1/1		1/1
Eyes -microphthalmia	1/1	1/1		
-anophthalmia				1/1[d]
total	1/1	1/1		2/2
Total with any malformation	3/3	4/3	0/0	2/2

[a]data excerpted from table 5 of submitted study, values confirmed by reviewer from individual animal data.

[b]number of fetuses/litters

[cde]signifies findings observed in the same fetus.

C.(2) Fetal Variations- Variations in development were tabulated by the investigators based on two criteria: (1) "developmental variations (minor differences from the normal developmental pattern for the species which are frequently observed in control specimens)" and (2) variations associated with delayed development. The only developmental variation (as defined by the investigators) noted was wavy ribs- 2 fetuses from one litter of the 300 mg/kg group and one fetus from the 1000 mg/kg group exhibited this variation.

Variations attributed to delayed development occurred mainly in the skeleton due to incomplete ossification, although 4/171 fetuses (3/24 litters) from the control group had slightly dilated lateral ventricles of the brain and 2/180 and 1/170 fetuses (1/25 litters each) from the 300 and 1000 mg/kg groups, respectively, exhibited this variation.

The incidence of delayed development of the skeleton appeared to be inversely dose-related. That is, the highest incidence of skeletal variations occurred in the low dose group, whereas the high dose group actually had a decreased incidence of this variation compared to control. This pattern occurred for several different ossification sites, notably hyoid, sternebrae, and xiphoid, and was reflected by the total % of fetuses and litters that were affected (table 5). Although delayed ossification was noted at several sites, only those sites which were affected in a significant number of animals were tabulated.

Table 5. Incidence of Delayed Ossification[a]

Site	Dose (mg/kg) 0	300	1000	3500
Number examined	181/24[b]	193/25	177/25	174/24
Skull -parietals	12/8	20/11	11/5	12/8
Hyoid	41/17	49/19	19/10	15/10
Sternebrae -unossified	59/20	81/24	64/21	39/16
Xiphoid -unossified	15/7	13/5	19/6	5/3
% Fetuses affected (affected/examined)	48.0 (87/181)	60.1 (116/193)	45.2 (80/177)	30.5 (53/174)
% Litters affected	95.8 (23/24)	100.0 (25/25)	88.0 (22/25)	79.2 (19/25)
Average % affected fetuses/litter	24.6±13.1	31.2±14.2*	22.4±13.5	16.4±13.7**

[a]data and statistics excerpted from table 6 of submitted study.

[b]number of fetuses/number of litters

*$p \leq 0.05$, **$p \leq 0.01$ by Mann-Whitney U Test.

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Conclusion

Treatment of pregnant rats with chloroneb at doses of 300, 1000 and 3500 mg/kg by oral gavage did not have any significant effects on fetal survival, fetal body weights, or the number of live fetuses per litter. Overt maternal toxicity was noted in mid and high dose dams as evidenced by increased incidence of physical signs. Dose-related decreases in maternal weight gain were also noted in treated animals during gestation.

No treatment-related malformations were noted.

A apparent dose-related effect on delayed skeletal ossification was noted in fetuses from mothers treated with the test compound. The dose-effect relationship for this phenomenon was inverse, i.e. the effect was greatest in the low dose group, whereas the high dose group had a statistically significant decrease in the incidence of delayed skeletal ossification relative to control. Although unusual for a study of this type, an inverse dose-effect relationship is not unprecedented as a pharmacological response to xenobiotics. Therefore, this reviewer cannot agree, at this time, with the conclusions of the investigators that "the average percentage of fetuses with any variation observed per litter was not dosage-dependent". The observed effect clearly was dose-dependent, and was linearly related (when plotted as a log dose-effect relationship, r= -0.999 for total % affected fetuses, r= -0.963 for average % affected fetuses/litter, and r= -0.995 for % affected litters, see appended graph). Because of the apparent linearity and dose-dependency of this effect, a NOEL for fetal skeletal variations cannot be established by these data at this time.

This unusual result may be related to the schedule of sacrifice, and thus insignificant, since fetal ossification is known to occur around day 20 of gestation. Because of the unusual nature of these findings the following information is requested in order to complete the evaluation of this study: (1) the order of sacrifice of treatment groups, (2) individual animal records of dose preparation and administration, (3) historical control data for the incidence of variations and malformations in this strain of rats at the contracting laboratory. Historical data for a period of at least two years before the present study, tabulated by fetal and litter incidence, is requested. Any other explanations or data relevant to this effect are also requested from the registrant.

Not a teratogen at the HDT (3500 mg/kg gavage).

NOEL, LEL for maternal toxicity will be determined after requested data is provided.

NOEL, LEL for fetal toxicity will be determined after requested data is provided.

Classification: Core-Supplementary Additional information (as specified above) is requested from the registrant as to the unusual effect of treatment on delayed fetal ossification that was noted in the study.

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Study: Preliminary Study for Embryo-Fetal Toxicity and Teratogenicity Study of Chloroneb in the Rat.

Accession No.: 251459

Study No.: 104-004P

Sponsor/Contracting Lab.: DuPont/Argus Research Laboratories

Report Date/Submitted: 6-24-83/10-3-83

Test Material: Chloroneb; purity and lot nos. not given.

Doses tested: 0, 300, 1000, and 3000 mg/kg/day by gavage.

Methods

This study was a range-finding study for the main study (Segment II) previously reviewed. The protocol for compound administration was stated to be the same for both studies. The protocol for measurement of animal data in the range-finding study was not described. It is assumed, based on the data provided, that the methods for animal measurements were similar in both studies. The following point is noted:

1) The study was intended as range-finding only, not for assessment of teratogenic potential.

Results

A. Clinical Signs and Mortality- One rat of the 300 mg/kg group died as a result of an apparent intubation error. No other deaths were reported.

A significant increase in the incidence and duration of excess salivation was noted in all treatment groups: 1/6 control, 5/6 low dose, 6/6 mid dose, and 6/6 high dose animals exhibited this sign. The duration (number of days) of this sign was also increased in a dose-related fashion. Urine-stained fur and red exudate in the inguinal region were also seen in the high dose group only.

B. Maternal Weight Gain- No significant effect of the test article on maternal weight gain during gestation was noted. All animals showed a slight decrease in weight gain over days 5-8 (dosing initiated on day 5), however by the end of the dosing period (day 14) no significant differences in body weight gain were apparent.

No effect of the test article on food consumption over days 0-5, 5-15, or 15-20 was noted. Consumption in test animals was similar to control over all of these intervals.

Body weight data are presented in table 1.

Table 1. Effect of Treatment on Maternal Body Weight Gain[a]

Days	Dose (mg/kg) 0	300	1000	3000
5-14	36.3±13.3	28.0±21.4	44.5± 8.7	31.0±20.4
5-20	113.8± 6.4	100.0±21.3	130.2±11.7	112.7±15.6
0-20	137.3± 6.9	129.0±25.2	155.8±16.5	146.2±17.1

[a]means ± standard deviation, data excerpted from table 2 of submitted study.

C. Reproductive Data- No effects of the test article on fertility, resorptions, litter size, or fetal birth weight were noted. These data are tablulated below.

Table 2. Reproductive and Litter Data[a]

Parameter	Dose (mg/kg) 0	300	1000	3000
No. litters/ mated females	6/6	6/6[b]	6/6	6/6
Total #fetuses	83	76	87	81
#dead fetuses	0	0	0	0
#live/litter	13.8±0.8	12.4±2.3	14.5±1.0	13.5±1.6
Mean birth weight (g)	3.7±0.1	3.8±0.3	3.7±0.3	3.6±0.3
Mean number corpora lutea	16.8±1.5	14.6±2.3	16.5±1.0	16.8±1.8
Mean number implantations	14.8±0.8	13.4±2.2	14.8±1.2	14.8±1.7
Mean number resorptions/litter	1.0±0.6	1.0±1.2	0.3±0.5	1.3±0.5
(mean %)	(6.7±4.2)	(7.2±8.7)	(2.2±3.3)	(9.0±3.3)

[a]data excerpted from submitted study. Values are mean ± std. dev., except where noted.

[b]one pregnant dam died on day 12 as a result of intubation error, therefore only 5 litters were delivered for this group although 6 females were pregnant.

D. Fetal Malformations and Variations- No compound-related visceral or skeletal malformations were noted in the low or mid dose groups. Fetuses from high dose dams were not examined for visceral or skeletal malformations or variations. No externally-visible malformations were noted in the high dose group.

One control fetus had hydronephrosis, and one fetus from the low dose group had four vessels arising from the aortic arch (in contrast to the normal three). One fetus from the mid dose group had a missing tail, clubbed forepaws (syndactyly), and fused lumbar vertebrae.

The only developmental variation noted was "slightly dilated" ureters in one fetus of the mid dose group, however high dose fetuses were not examined.

Variations classified by the investigators as "retarded development" were noted only in the skeleton, due to ossification delay. No significant effect of the test compound on the overall incidence of skeletal ossification delay was apparent. However, since high dose fetuses were not examined, a valid assessment of this or any other potential fetotoxic effects is not possible based on this study.

Conclusions

This study was intended as a range-finding study for a teratology study with chloroneb. Based on the results of this study, the doses used in the main teratology study appear to be reasonable.

Under the conditions of this study, no effects of the test compound on maternal weight gain or reproductive parameters were noted. Dose-related increases in the incidence and duration of salivation and stained abdominal fur were noted in treated dams on physical examination.

Litter parameters were also unaffected by treatment. Litter size, fetal body weight, and the incidence of malformations and variations were not altered by the test compound. However, the small number of dams placed on test and the lack of assessment of high dose fetuses limits the utility of these data.

Classification: Core-Supplementary For range-finding purposes only.

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Study Title: Three-Generation Reproduction Study

Accession No.: 251458

Sponsor/Contracting Lab.: DuPont/Hazelton Laboratories

Report Date/Submitted: 5-24-67/10-3-83

Test Compound: Fungicide 1823, 75% WP weeks 0-34, 65% WP weeks 35-88.

Doses Tested: 0, 100, and 500 ppm in feed.

Methods

A photocopy of the submitted methods is appended below.

Procedure Fungicide 1823 was fed to male and female albino rats of the Charles River Caesarean-derived strain, starting with approximately 25-day-old animals, for 14 weeks, at levels of 0.0, 0.01, and 0.05% of a nutritionally complete diet (Purina Laboratory Chow).

After completion of the feeding period, a reproduction study was conducted with 10 male and 20 female rats within each group, in which F_{1A} and F_{1B} litters were cast. Ten male and 20 female weanling rats were selected from the F_{1B} litter in each group and continued on their respective diets for approximately 100 days, at which time they were bred within each group to produce F_{2A} and F_{2B} litters. This same procedure was followed with the F_{2B} litter to produce the F_{3A} and F_{3B} litters. Ten male and 10 female weanling rats of the F_{3B} litter from the control and each test group (0.01 and 0.05%) were subjected to a histopathological evaluation, following gross necropsy.

The following deficiencies are noted:

1) Only two dose levels used.

2) Treatment with the test article was apparently discontinued during mating, gestation, and lactation.

3) No data for weight change of males or females during treatment.

4) No tabulations of lesions or individual animal necropsy data.

5) Method of sacrifice of animals not described.

Results

A. Mortality- Specific mortality data were not submitted. Based on submitted clinical sign data, apparently no dams from any of the generations died during the course of the study.

B. Clinical signs- No clinical signs were noted in dams that could be related to treatment. Common signs observed in animals from all groups included nasal discharge, stains/bloody crust on nose, wheezing, and rough haircoat. Clinical signs were submitted for some pups, however data were not submitted for every litter, and it was therefore not possible to assess these data for possible treatment-related effects.

C. Reproductive data- (1) Fertility- No effect on fertility was noted as a result of treatment with the test article. Similar numbers of dams were pregnant in all groups and generations (90-100%).

(2) Maternal weight change- No useful data were submitted for this parameter. A table was submitted for each mating which contained two weights for each animal, however the weights were not all taken on the same day for animals of the same mating. Further, each table bore the subscript "based on estimated data". The basis for estimating these numbers was not defined. No other parental body weight data were submitted.

D. Litter data- (1) Litter size- No effect of the test article on litter size (number of live pups/litter, table 1), or on the number of pups dead at birth (number/litter or % affected litters) was noted. No effect on sex ratio (% male pups) was apparent.

Table 1. Litter Size[a]

Mating	0 ppm	100 ppm	500 ppm
F_{1a}	10.9 ± 3.0[b]	11.7 ± 3.2	10.1 ± 3.9
F_{2a}	11.4 ± 2.7	11.9 ± 2.8	11.6 ± 2.7
F_{3a}	10.5 ± 3.5	12.9 ± 2.1	11.3 ± 2.4
F_{1b}	11.1 ± 4.3	12.5 ± 2.8	11.6 ± 3.1
F_{2b}	13.1 ± 2.1	13.7 ± 2.9	13.0 ± 2.7
F_{3b}	10.0 ± 3.6	12.9 ± 2.4	12.1 ± 3.1

[a]data excerpted from submitted raw data.

[b]mean ± std. dev., calculated by reviewer from submitted raw data.

(2) Fetal birth weights- The birth weights of pups was calculated by the reviewer from submitted raw data (table 2). Pups from each litter were evidently separated by sex and weighed as a group. The group birth weight was divided by the number of pups to obtain an average individual fetal birth weight, by sex, for each litter. No effect of the test article on fetal birth weights was noted.

Table 2. Fetal Birth Weights[a]

Mating	0 ppm		100 ppm		500 ppm	
	Male	Female	Male	Female	Male	Female
F_{1a}	7.35±0.77	7.17±0.99	7.16±0.82	6.74±0.72	7.33±0.89	7.18±0.93
F_{2a}	6.91±0.73	6.68±0.66	6.84±0.72	6.66±0.57	6.98±0.67	6.72±0.69
F_{3a}	7.36±1.03	7.09±0.95	6.65±0.64	6.76±0.97	7.40±0.76	7.14±0.64
F_{1b}	7.65±0.80	7.04±0.81	7.23±0.75	6.87±0.69	7.15±0.64	6.71±0.78
F_{2b}	6.85±0.69	6.74±1.28	6.95±0.72	6.54±0.75	7.25±0.75	6.73±0.52
F_{3b}	7.23±0.71	6.99±1.12	6.72±0.49	6.54±0.79	7.29±0.80	7.00±0.73

[a]data excerpted from submitted raw data. Values are mean ± std. dev., calculated by reviewer.

(3) Survival to weaning- Litter sizes were reduced on day 1 to 8 pups/litter. Where possible the sex ratio was maintained at 50% males at culling. Survival of culled pups to day 21 was calculated by the reviewer based on submitted raw data. Low survival was noted in all pups of the F_{1a} mating; no explanation for this effect was provided in the submitted data. The F_{2a} and F_{3a} matings did not exhibit this high rate of mortality. The only group in which a decrease in % survival was noted that could be related to treatment was the 500 ppm group from the F_{3b} generation (table 3). The incidence of pup deaths was correspondingly increased in this group, both as the number of dead fetuses/litter and as the % affected litters (table 4). An increase in the % of litters with a dead pup was also noted in the high dose group of the F_{3a} mating, although no increase in the number of dead pups/litter was apparent.

Table 3. Pup Survival To Day 21[a]

Dose (ppm)	Mating F1a	F1b	F2a	F2b	F3a	F3b
0	65.5	89.8	98.7	95.1	97.9	95.9
100	63.8	95.2	93.2	99.3	97.5	97.5
500	59.6	97.9	95.5	97.4	93.6	83.8

[a]data excerpted from submitted raw data. Values are percent of pups (post-cull) that survived to day 21, calculated by reviewer.

Table 4. Incidence of Pup Deaths By Day 21[a]

Dose (ppm)	Mating F1a	F1b	F2a	F2b	F3a	F3b
0	2.63[b] (68.4)[c]	0.78 (22.2)	0.10 (10.0)	0.39 (27.8)	0.15 (15.0)	0.30 (20.0)
100	2.75 (85.0)	0.37 (15.8)	0.53 (15.8)	0.05 (5.3)	0.20 (15.0)	0.20 (15.0)
500	3.11 (78.9)	0.17 (11.1)	0.35 (15.0)	0.20 (15.0)	0.50 (38.9)	3.00 (42.1)

[a]data excerpted from submitted raw data.

[b]number of pups/litter that died by day 21 (after culling), calculated by reviewer.

[c]percent of litters with one or more pups that died by day 21 (after culling), calculated by reviewer.

E. Necropsy Data: No tabulations or individual animal data were submitted for either gross or histopathological examinations. The final report stated that "no compound-related gross or histopathologic changes were observed in any tissues examined from the weanling rats." Tissues that were examined included brain, spinal cord, eye, pituitary, thyroid, thymus, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, small intestine, large intestine, urinary bladder, gonad, bone (rib junction), and sternal bone marrow (list copied from final report narrative).

Weights of heart, liver, spleen, kidney, testes, thymus, brain, and lungs were reported for 10 fetuses of each sex for animals of one of the matings of the F_3 generation (which one was not reported). The age of these animals at necropsy was not clear. The submitted individual animal (computer print-outs) data indicated that rats were 3 weeks of age. The body weights of these animals, as tabulated on the organ weight data sheets, was 70-80 grams on average. However, day 21 body weights calculated by the reviewer from individual animal survival-to-weaning data resulted in an average body weight of about 50 grams for animals of this and other generations. Therefore, although an approximately 25% decrease in the relative organ weight of spleen was noted in high dose females, the interpretation of these data is uncertain due to confusion as to the age of the pups and the lack of correlative necropsy data for these animals.

Conclusions

No effects of the test article on fertility, litter size, or birth weight were noted. The only potential effect of the test article was slightly decreased survival of pups to day 21 in the high dose group (500 ppm) of the F_{3b} mating. No individual animal data for necropsy of pups (other than organ weights) were submitted. No other useful data for gross or histopathological examinations of pups were submitted.

Insufficient data were submitted for assessment of parental toxicity. Although no treatment-related clinical signs were noted, no data on the effect of treatment with the test article on weight change in either male or female rats were submitted. The data submitted for maternal weight change during gestation all contained the subscript "based on estimated data". This subscript is meaningless to this reviewer. In any case, only two weights were reported for each dam over the course of gestation, all on different days so that no comparison of treatment groups was possible even based on "estimated data". No individual animal or summary tabulations of necropsy data were submitted.

Several other deficiencies in the study were noted. Only two dose levels were used, instead of three as required for Core-Minimum data. The submitted description of methods (although incomplete) implies that dosing was discontinued during mating, gestation, and lactation, periods of experimental interest in a reproduction study.

Data are inadequate for the establishment of a reproductive NOEL.

Classification: Core-Supplementary Only two dose levels used, inappropriate dosage schedule, inadequate description of methods, inadequate reporting of animal data, no apparent maternal toxicity at the HDT.

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