micrfiche

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

010498

AUG 17 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 041701. Fonofos (Dyfonate®). Rereview of Multigeneration Reproduction Study

Shaughnessy No. 041701
Tox. Chem. No. 454B

TO: Rick J. Whiting
Peer Review Section
Science Analysis Branch
Health Effects Division (H7509C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 8/6/93
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 8/6/93 KB 8/10/93
Section I, Toxicology Branch I
Health Effects Division (H7509C)

The multigeneration reproduction study conducted on Fonofos was rereviewed in preparation for the RfD Committee review for the corn cluster project. The study is classified as Core Supplementary and does not satisfy the regulatory requirements for a reproduction study in rats. The Data Evaluation Record (DER) is attached. The following is a summary of the results of the study.

Dyfonate was tested in a 3-generation reproduction study in rats at 0, 10.0 or 31.6 ppm in the diet. The F_0 parents received one-half the respective dose for the first 4 weeks and the F_2 parents received one-half the respective dose for the first week. According to the available data, no effects were observed for either parental systemic toxicity or for reproductive parameters at either dose level. In addition, no effects were observed for pup body weights or viability during lactation. However, deficiencies in the study prevent an adequate assessment of parental toxicity or reproductive effects. Therefore, an accurate NOEL cannot be estimated.

1522

Reviewed by: Pamela M. Hurley, Toxicologist Pamela M. Hurley 5/21/93
Section I, Toxicology Branch I (H7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Toxicology Branch I (H7509C) Roger Gardner 5-21-93

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

TOX. CHEM. NO./SHAUGHNESSY NO.: 454B/041701

MRID NUMBER: 00082234

DP BARCODE/SUBMISSION NO.: N/A

TEST MATERIAL: Fonofos

SYNONYMNS: Dyfonate; N-2790

STUDY NUMBER(S): Not available

SPONSOR: Stauffer Chemical Company

TESTING FACILITY: Woodard Research Corporation (no address on study)

TITLE OF REPORT: Dyfonate (N-2790) Three-Generation Reproduction Study in Rats

AUTHOR(S): M. Woodard, C. L. Leigh, G. Woodard

DATE REPORT ISSUED: 1/10/69

CONCLUSIONS: Dyfonate was tested in a 3-generation reproduction study in rats at 0, 10.0 or 31.6 ppm in the diet. The F_0 parents received one-half the respective dose for the first 4 weeks and the F_2 parents received one-half the respective dose for the first week. According to the available data, no effects were observed for either parental systemic toxicity or for reproductive parameters at either dose level. In addition, no effects were observed for pup body weights or viability during lactation. However, deficiencies in the study prevent an adequate assessment of parental toxicity or reproductive effects. Therefore, an accurate NOEL cannot be estimated.

CLASSIFICATION: Core Supplementary

TESTING GUIDELINE SATISFIED: None

I. PROTOCOL

A. Materials

1. Test Material:

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: Clear liquid
Batch #(s), Other #(s): Lot HMP 25, 4/12/66
Purity: 99.8 - 99.9%
Source: Stauffer Chemical Company
Vehicle (if applicable): acetone (evaporated off during mixing of diet)

2. Test Animals:

Species and Strain (sexes): Male and female CD albino rats (random bred Sprague-Dawley descendents).

Age: 32 days upon receipt, 100 days at mating
Weight(s): Mean approx. 100 g (♂), Mean approx. 90 g (♀) at week 0 of the study.
Source(s): Charles River Breeding Laboratories, Inc. (address not given).

The rats were acclimated for a period of 11 days before they were placed into the study.

3. Diet preparation: The diets were prepared by adding 20 ml of a solution of Dyfonate in acetone to rat chow and mixed in a Hobart mixer. A separate solution was prepared for each test group. The acetone was evaporated from the diet in the process of mixing.

Frequency of preparation: Not stated.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Not conducted.

B. Procedures and Study Design

1. Mating: 1 male was caged with 1 female (in several cases in each dose group, 1 male was paired with 2 females) from the same test group until a vaginal plug was observed, indicating that mating had taken place. If the vaginal plug was not found after 10 days' observation, the first male was removed and a few days later was replaced by another male from the same test group. It was not stated what was done if two attempts at mating were unsuccessful.

2. Mating schedule: The F_0 parental animals were given test diets for 68 days before they were mated, the F_1 parental animals were not mated until 45 days after they were selected from the F_{1b} litters, and the F_2 parental animals were not mated until 62 days after they were selected from the F_{2b} litters. Selection of parents for the F_1 generation was made when the pups were 24 - 47 days of age and selection of parents for the F_2 generation was made when the pups were 36 - 44 days of age. The mated animals in the study were approximately 100 days (F_0, first litters), 69 - 92 days (F_1, first litters) and 98 - 106 days (F_2 first litters) of age at mating.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per group[1]	
No.	Designation	(ppm) [2]	Males	Females
1	Control	0	20	22
2	Low (LDT)	5.0 (4 wks) 10.0 (wks 5 & up)	20	22
3	High (HDT)	15.8 (4 wks) 31.6 (wks 5 & up)	20	22

[1]22 - 24 males and 21 - 23 females were selected for the F_1 parents and 20 males and females were selected for the F_2 parents.

[2]Diets were administered from the beginning of the study until the animals were sacrificed.

[3]The F_1 parents were fed 10.0 and 31.6 ppm Dyfonate in the diet from the time they were weaned and selected for the study until they were sacrificed. The F_2 parents were fed 5.0 and 15.8 ppm of the diet for the first week after they were weaned and selected for the study. They were then fed 10.0 and 31.6 ppm, respectively for the remainder of the study.

C. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity		Daily during premating and growth periods.
Detailed clinical observations		Once a week during growth and breeding periods.
Body weight	All animals	At beginning of study and biweekly through growth except during mating periods.
	Maternal animals	Not weighed during gestation and lactation in order to avoid undue handling.
Food consumption		Weekly during premating period.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study. A mating was considered successful if implantation sites were observed. Results were presented on the number of litters produced per dose level (either 0, 1 or 2) for each generation. Data on uterine implantation sites were also presented. In addition, the gonad weights were also discussed in this section. The fertility indices were not calculated in the report.

3. Litter observations: According to the report, the following litter observations were made:

Observation	Time of observation (lactation day) Birth	Day 4	Day 11	Day 21	Day 29
Number of live pups	x	x		x	
Pup weight (mean litter wt.)	x	x		x	
External alterations	x			x	
Number of dead pups	x			x	
Sex of each pup					

Gestation and viability indices were not provided in the report.

4. Necropsy

a. Parental animals: All surviving parental males and females were sacrificed one month (F_0), 16 - 23 days (F_1) and a few days (F_2) after the last litters in each generation were weaned. Gonads were weighed and uterine implantation sites were counted. These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead	Not stated in report	
Unscheduled sacrifice	Not stated in report	
Scheduled sacrifice	x	some saved

b. Offspring: The F_1, F_2 and F_3 offspring were sacrificed at birth (the F_{1a} and half of the F_{2a} litters), at day 4 (culled pups) and at day 21 (unused weanlings). These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead	x	visceral & skeletal[1]
Scheduled sacrifice	x	visceral & skeletal[2]

[1]One quarter of each F_{2a} litter and dead pups at birth, stillborn F_{1b} generation pups and F_{1b} pups that died prior to weaning were cleared, stained and examined for skeletal changes. A second quarter of each F_{2a} litter was preserved and examined grossly. The other half of each F_{2a} litter were not sacrificed and examined until weaning.

[2]Although some tissues were saved for possible future microscopic examination, actual examinations were only conducted on F_{3b} weanlings: from 1 male and 1 female from each litter.

c. Necropsy observations: Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues were prepared for microscopic examination:

x Liver	x Adrenals
x Kidneys	x Thyroid
x Heart	x Gonads
x Spleen	x Bone marrow

The gonads of males and females in the F_0 generation that either sired or produced no litters were preserved for possible future microscopic examination. In actuality, these were not examined.

D. Statistical analyses: The report stated that "a computerized analysis of variance was used for comparing absolute ovary weights of the treated groups with those of the control group. The analysis of variance was followed by Duncan's Range Test."

II. REPORTED RESULTS

A. Analysis of test diets: These analyses were not conducted.

B. Parental animals

1. Mortality and clinical signs:

F_0 Generation: At the 31.6 ppm dose level, 2 females and 1 male died during the study. The male died during week 28, following a marked body weight loss. One female died a few days after being paired for mating (no vaginal plug was found), and the other female was found dead during week 23. She produced 9 pups during the first mating and no pups during the second mating. No other parental rats from the F_0 generation died during the study. Records of clinical signs of toxicity were not provided in the report, although it was stated that the animals were observed daily for general appearance and behavior.

F_1 Generation: Five control (2 males and 3 females) and three 10.0 ppm male rats either died or were sacrificed in moribund condition during the study. In the controls, 1 male had marked body weight loss prior to death and the other had a possible stomach ulcer. One female had purulent exudate from both ears. In the 10.0 ppm group, two had marked body weight loss prior to death and blood found in either the

peritoneal cavity and/or gastrointestinal tract. No other parental animals died in the F_1 generation.

F_2 Generation: In the 31.6 ppm dose group, 1 male and 2 females died during the course of the study and in the 10.0 ppm dose group, 1 male died during the course of the study. At the 31.6 dose level, 1 animal had body weight loss prior to death and another was found emaciated. In the 10.0 ppm dose group, the animal was found emaciated and with an unthrifty appearance. No other parental animals died in the F_2 generation.

2. Body weight and food consumption: Body weights were measured biweekly over the entire study, except during the mating periods for males and during the mating, gestation and lactation periods for females. These were not separated out and it is difficult to tell when the pre-mating period for the second set of litters begins. The lack of specific body weight measurements during the pre-mating, gestation and lactation periods, particularly for the F_b litters, is a major deficiency for this study. It will be difficult to use body weights as a measurement of sufficiency of dose levels for this study.

F_0 generation: No treatment-related effects were observed for mean body weights or food consumption over the entire study for the times in which they were measured.

F_1 generation: No treatment-related effects were observed for mean body weights or food consumption over the entire study for the times in which they were measured.

F_2 generation: No treatment-related effects were observed for mean body weights or food consumption over the entire study for the times in which they were measured.

Reported body weight results are summarized as follows:

Selected Mean Body Weight Data For Males and Females

Dietary Level		Mean Body Weight in Grams				
		F_0 Generation				
		Males				
Group	ppm	Week 0	Week 8	Week 13	Week 21	Sac.
I	0	99	402	445	522	519
II	10.0	97	396	447	524	517
III	31.6	101	401	426	512	511
		Females				
Group	ppm	Week 0	Week 8	Week 15	Week 23	Sac.
I	0	88	238	276	291	271
II	10.0	92	246	285	308	283
III	31.6	87	236	288	311	281
		F_1 Generation				
		Males				
Group	ppm	Week 0	Week 8	Week 13	Week 21	Sac.
I	0	117	356	400	453	456
II	10.0	97	342	376	447	449
III	31.6	129	369	420	471	469
		Females				
I	0	98	232	233	272	267
II	10.0	88	226	229	284	268
III	31.6	98	231	227	273	274
		F_2 Generation				
		Males				
I	0	132	383	454	520	537
II	10.0	131	372	453	484	504
III	31.6	129	376	458	491	540
		Females				
I	0	105	220	288	376	297
II	10.0	103	217	285	332	299
III	31.6	109	215	286	339	290

3. Reproductive performance:

As stated in the procedural section, the results were reported as the distribution of litters per female. There were no data stating how many females successfully mated (i.e. vaginal plugs) in comparison as to how many produced litters. The number that were pregnant can be approximated by the number of implantation sites, however, one cannot accurately tell from which litter the implantation sites were from. Therefore, neither the mating, fertility or gestation indices could be estimated. The results below were calculated by the reviewer from any available individual animal data.

Reproductive Parameters

Observation	Dose Group			
	Control	Low	Mid	High
F_0 Generation - Litter A				
Median precoital interval (days)	Not available			
Males				
Number paired	18	14[1]		16
# Siring litters	13	12	-	13
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	72.2%	85.7%		81.3%
Females				
Number paired	22	22		21
Intercurrent deaths	0	0	-	0
Number giving birth	16	15		17
# litters/# ♀ paired x 100	72.7%	68.2%		81%
Median gestation interval (days)	Not available		-	
Number of litters (day 1)	16	15	-	17
Total litter losses	Not available			
Mean pup male/female ratio[2]	Not available			
F_0 Generation - Litter B				
Males				
Number paired	17	18		17
# Siring litters	16	14	-	16
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	94.1%	77.8%		94.1%

Reproductive Parameters

Observation	Dose Group Control	Low	Mid	High
Females				
Number paired	22	22		21
Intercurrent deaths	0	0	-	1
Number giving birth	20	16		18
# litters/# ♀ paired x 100	91.0%	72.7%		85.7%
Number of litters (Day 1)	20	16	-	18
Mean uterine implantation sites/dam for 2 litters	17.8	14.3	-	18.5
Mean # Litters produced/female				
0	1	4		1
1	7	5	-	5
2	14	13		15

[1]In examining the individual animal data, it is apparent that some of the males were not mated in both litters.
[2]The sex of specimens examined for visceral changes/group were determined from part of the F_{2a} litters. They are as follows: control: 16 males, 17 females; 10.0 ppm: 17 males, 17 females; 31.6 ppm: 18 males, 15 females. Three controls, one 10.0 ppm and eight 31.6 ppm pups were excluded due to mutilation and autolyzation. It was not stated how these animals were selected. Therefore, the numbers of males and females does not necessarily reflect the ratios of males to females that were actually present in the litters.

Reproductive Parameters

Observation	Dose Group Control	Low	Mid	High
F_1 Generation - Litter A				
Males				
Number paired	23	21		22
# Siring litters	18	16	-	17
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	78.3%	76.2%		77.2%
Females				
Number paired	23	21		22
Intercurrent deaths	0	0	-	0
Number giving birth	18	16		17
# litters/# ♀ paired x 100	78.3%	76.2%		77.2%
Number of litters (Day 1)	18	16	-	17

Reproductive Parameters

Observation	Dose Group Control	Low	Mid	High
F_1 Generation - Litter B				
Males				
Number paired	22	20		23
# Siring litters	16	16	-	22
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	72.7%	80.0%		95.7%
Females				
Number paired	22	21		23
Intercurrent deaths	0	0	-	0
Number giving birth	16	16		22
# litters/# ♀ paired x 100	72.7%	76.2%		95.7%
Number of litters (Day 1)	16	16	-	22
Mean uterine implantation sites/dam for 2 litters	Not available	-	-	-
Mean # Litters produced/female				
0				
1	1	2		1
2	10	6	-	5
	12	13		17

Reproductive Parameters

Observation	Dose Group Control	Low	Mid	High
F_2 Generation - Litter A				
Males				
Number paired	19	19		19
# Siring litters	14	16	-	18
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	73.7%	84.2%		94.7%
Females				
Number paired	19	19		19
Intercurrent deaths	0	0	-	0
Number giving birth	14	16		18
# litters/# ♀ paired x 100	73.7%	84.2%		94.7%
Number of litters (Day 1)	14	16	-	18

Reproductive Parameters

Observation	Dose Group: Control	Low	Mid	High
F_2 Generation - Litter B				
Males				
Number paired	19	19		16
# Siring litters	17	16	-	15
Intercurrent deaths	0	0		0
# litters/# ♂ paired x 100	89.5%	84.2%		93.8%
Females				
Number paired	19	19		17
Intercurrent deaths	0	0	-	0
Number giving birth	17	16		16
# litters/# ♀ paired x 100	89.5%	84.2%		94.1%
Number of litters (Day 1)	17	16	-	16
Mean uterine implantation sites/dam for 2 litters	19.7	20.8	-	22.3
Mean # litters produced/female				
0	2	0		0
1	3	6	-	4*
2	14	13		15

* Two females were mated only one time.

4. Necropsy results

a. Organ weights: It appears that the absolute ovary weights for both treated groups are statistically significantly smaller than the control group in the F_0 generation. Statistical analyses were not conducted on the relative weights since they were not calculated in the report. Ovary weights were not measured in any of the other generations. Mean testes and ovary weight results for male and female rats are summarized from the report as follows:

Observation	Dose group			
	Control	Low	Mid	High
F_0 Generation				
Testes weight (g)	3.52	3.32	-	3.43
Adjusted for body weight (g/g)	0.0068	0.0064	-	0.0067
Ovaries weight (mg)	88.2	76.0[2]	-	71.7[1]
Adjusted for body weight (mg/g)	0.325	0.269	-	0.253
F_1 Generation				
Testes and Ovaries weights (g)	Not available			
Adjusted for body weight (g)	Not available			
F_2 Generation				
Testes weight (g)	3.56	3.53	-	3.52
Adjusted for body weight (g)	0.0066	0.0070	-	0.0065
Ovaries weight (mg)	Not available			
Adjusted for body weight (mg/g)	Not available			

[1]ANOVA & Duncan's Range Test: statistically significant from control p = 0.01.
[2]ANOVA & Duncan's Range Test: statistically significant from control p = 0.05.

b. Pathology

i. Macroscopic examination: The report stated that parental rats were grossly examined. However, there were no tables or statements concerning what, if anything was observed.

ii. Microscopic examination: No microscopic examinations were conducted on the parental animals.

C. Offspring

1. Viability and clinical signs: In the F_1 litters, the report stated that minor cage injuries, mutilation, cannibalism and a few instances of lung congestion were observed. No tables were provided. Evidence of mutilation, cage injuries and cannibalism were also observed in the F_2 and F_3 litters. Again, no tables were provided. The authors stated that there were no treatment-related effects for clinical signs of toxicity, but had no data to support the statement. The report also stated that "several of the weanlings [in the F_3 generation] in both treated and control groups showed tail lesions caused by Myobia sp., so called 'ringtail'. This condition has been attributed to a low relative humidity."

There do not appear to be any treatment-related effects on viability, gestation and lactation indices. There also does not appear to be any treatment-related effect on litter size during lactation. In the F_3 generation, the controls lost 1-2 litters between days 4 and 21. Thus, the mean litter sizes appear to increase, creating an artifactual effect.

Viability results from pups during lactation are summarized from the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
F_1 Generation				
Litter A				
# Pups born alive	182	158	-	168
# Dead pups at birth	7	9	-	6
Gestation index[1]	96.3%	94.6%	-	96.6%
# Pups (day 4 before cull)	Not available			
# Pups (day 4 post-cull)	Not available			
Number of pups (day 21)	Not available			
Pup deaths (Days 1-21)	Not available			
Viability index (day 4)	Not available			
Lactation index (day 21)	Not available			
Litter B				
# Pups born alive	190	177	-	186
# Dead pups at birth	16[4]	2	-	14[4]
Gestation index	92.2%	98.9%	-	93.0%
# Pups (day 4 before cull)	152	164	-	150
# Pups (day 4 post-cull)	142	145	-	132
Number of pups (day 21)	115	126	-	116
Pup deaths (Days 1-21)	65	32	-	52
Viability index (day 4)[2]	80%	92.7%	-	80.6%
Lactation index (day 21)[3]	80.9%	86.9%	-	87.9%
F_2 Generation				
Litter A				
# Pups born alive	171	172	-	185
# Dead pups at birth	3	1	-	7
Gestation index	98.3%	99.4%	-	96.4%
# Pups (day 4 before cull	Cannot calculate - some sacrificed			
# Pups (day 4 post-cull)	78	70	-	87
Number of pups (day 21)	72	62	-	78
Pup deaths (Days 1-21)	20	27	-	25
Viability index (day 4)	Cannot calculate			
Lactation index (day 21)	92.3%	88.6%	-	89.7%

Observation and study time	Dose group Control	Low	Mid	High
Litter B				
# Pups born alive	149	158	-	216
# Dead pups at birth	3	1	-	14[5]
Gestation index	98.0%	99.4%	-	93.9%
# Pups (day 4 before cull)	135	145	-	194
# Pups (day 4 post-cull)	130	139	-	183
Number of pups (day 21)	121	129	-	158
Pup deaths (Days 1-21)	21	19	-	47
Viability index (day 4)	90.6%	91.8%	-	89.8%
Lactation index (day 21)	93.1%	92.8%	-	86.3%
F_3 Generation				
Litter A				
# Pups born alive	154	173	-	192
# Dead pups at birth	9	8	-	6
Gestation index	94.5%	95.6%	-	97.0%
# Pups (day 4 before cull)	142	129	-	163
# Pups (day 4 post-cull)	119	122	-	143
Number of pups (day 21)	103	89	-	136
Pup deaths (Days 1-21)	17	75	-	36
Viability index (day 4)	92.2%	74.6%	-	84.9%
Lactation index (day 21)	86.6%	73.0%	-	95.1%
Litter B				
# Pups born alive	186	182	-	189
# Dead pups at birth	15	18	-	10
Gestation index	92.5%	91.0%	-	95.0%
# Pups (day 4 before cull)	137	131	-	148
# Pups (day 4 post-cull)	121	116	-	127
Number of pups (day 21)	104	72	-	103
Pup deaths (Days 1-21)	66	92	-	65
Viability index (day 4)	73.7%	72.0%	-	78.3%
Lactation index (day 21)	86.0%	62.1%	-	81.1%

[1]Gestation index = $\frac{\text{\# live pups born}}{\text{\# live + dead pups born}} \times 100$

[2]Viability index = $\frac{\text{\# live pups at day 4}}{\text{\# pups born alive}} \times 100$

[3]Lactation index = $\frac{\text{\# live pups at day 21}}{\text{\# pups alive at postcull}} \times 100$

[4]Large number of presumed stillbirths produced was mainly due to 2 females in each of the control and high dose groups.

[5]12 of the 14 stillborns were from 1 female.

Changes in mean litter sizes were summarized in the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
F1 Generation				
Litter A				
Day 1	11.4	10.5	-	9.9
Day 4 pre-cull		Not available		
Day 4 post-cull		Not available		
Day 7		Not available		
Day 14		Not available		
Day 21		Not available		
Litter B				
Day 1	9.5	11.C	-	10.0
Day 4 pre-cull	8.94	10.9	-	10.0
Day 4 post-cull	8.35	9.67	-	8.8
Day 7		Not available		
Day 14		Not available		
Day 21	7.19	9.0	-	8.3
# Total litter losses (d 1-21)	4	2	-	4
F_2 Generation				
Litter A				
Day 1	9.5	10.7	-	10.9
Day 4 pre-cull	Cannot be calculated - some sacrificed			
Day 4 post-cull	4.9	5.0	-	5.4
Day 21	4.8	4.8	-	5.2
# Total litter losses (d 1-21)	3	3	-	2
Litter B				
Day 1	9.3	9.9	-	9.8
Day 4 pre-cull	8.4	9.1	-	9.2
Day 4 post-cull	8.1	8.7	-	8.7
Day 21	8.1	8.1	-	7.9
# Total litter losses (d 1-21)	1	0	-	2
F_3 Generation				
Litter A				
Day 1	11.0	10.8	-	10.7
Day 4 pre-cull	10.9	8.6	-	9.6
Day 4 post-cull	9.2	8.1	-	8.4
Day 21	9.4	7.4	-	8.0
# Total litter losses (d 1-21)	1	4	-	1
Litter B				
Day 1	10.9	11.4	-	11.8
Day 4 pre-cull	9.1	9.4	-	9.9
Day 4 post-cull	8.1	8.3	-	8.5
Day 21	8.7	7.2	-	7.9
# Total litter losses (d 1-21)	5	6	-	3

2. Body weight: There does not appear to be any treatment-related effect on mean pup body weights during lactation. Selected group mean body weights (g) are summarized from the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
F_1 Generation				
Litter A				
Mean pup weight (day 1)	5.5	5.4	-	5.8
Mean pup weight (day 4 pre-cull)		Not available		
Mean pup weight (day 7)		Not available		
Mean pup weight (day 14)		Not available		
Mean pup weight (day 21)		Not available		
Weight gain: days 1 - 21		Not available		
Litter B				
Mean pup weight (day 1)	6.5	6.5	-	6.2
Mean pup wt (day 4 pre-cull)	9.8	9.7	-	9.2
Mean pup weight (day 7)		Not available		
Mean pup weight (day 14)		Not available		
Mean pup weight (day 21)	39.5	38.5	-	41.0
Mean Weight gain: days 1 - 21	33.0	32.0	-	34.8
F_2 Generation				
Litter A				
Mean pup weight (day 1)	5.6	5.2	-	5.2
Mean pup wt (day 4 pre-cull)	9.3	8.8	-	8.7
Mean pup weight (day 21)	42.5	39.1	-	41.4
Mean Weight gain: days 1 - 21	36.9	33.9	-	36.2
Litter B				
Mean pup weight (day 1)	6.7	6.0	-	6.3
Mean pup wt (day 4 pre-cull)	11.4	10.6	-	11.1
Mean pup weight (day 21)	38.2	41.4	-	39.9
Mean Weight gain: days 1 - 21	31.5	35.4	-	33.6
F_3 Generation				
Litter A				
Mean pup weight (day 1)	6.6	6.5	-	6.5
Mean pup wt (day 4 pre-cull)	10.1	10.2	-	10.1
Mean pup weight (day 21)	39.9	36.7	-	35.6
Mean Weight gain: days 1 - 21	33.3	30.2	-	29.1
Litter B				
Mean pup weight (day 1)	6.2	5.9	-	6.1
Mean pup wt (day 4 pre-cull)	9.1	7.8	-	7.7
Mean pup weight (day 21)	34.9	34.4	-	33.2
Mean Weight gain: days 1 - 21	28.7	28.5	-	27.1

3. Necropsy results

a. Organ weights: Organ weights from the liver, kidney and heart were determined for the F_{3b} weanling rats. There were no treatment-related effects. These results are summarized from the report as follows:

Mean Body Weight and Absolute and Relative Organ Weights (g) for F_{3b} Weanlings

Level (ppm)	Body Weight	Liver	Kidney	Heart
		Males		
Control	39	1.81 (0.046)*	0.56 (0.014)	0.26 (0.007)
10.0	37	2.02 (0.055)	0.57 (0.015)	0.23 (0.006)
31.6	33	1.57 (0.048)	0.51 (0.015)	0.22 (0.007)
		Females		
Control	33	1.78 (0.054)	0.57 (0.017)	0.27 (0.008)
10.0	37	1.72 (0.046)	0.58 (0.016)	0.22 (0.006)
31.6	31	1.64 (0.053)	0.54 (0.017)	0.23 (0.007)

* Absolute value (relative value)

b. Pathology

i. Macroscopic examination:

F_1 Pups: The report noted that gross observations of the F_1 pups at birth and weaning showed no malformations. Skeletal examination of stillborn pups and those which died prior to weaning showed no developmental effects or changes in the ossification rate. In all, 21, 6 and 27 fetuses were examined for skeletal effects from the control, low dose and high dose groups, respectively.

F_2 Pups: Skeletal examination of one-half of each F_{2a} litter showed no developmental effects or changes in the ossification rate as well. For these, 37, 43 and 43 pups were examined for the controls, low dose and high dose groups, respectively. The table for these examinations is provided below. Gross examinations of the F_2 pups at various time points indicated no treatment-related effects. The gross examination tables to support these statements were not specifically provided in the report (a table was provided, but it only showed how many were examined). The report stated that "one weanling from each of 4 different 10.0 ppm litters showed a small mass on the right eyelid, a small, hard and reddened testis, a protrusion of the umbilicus and concretions in the urinary bladder, respectively."

F_3 Pups: No malformations were observed in the F_3 litters. No other gross lesions were observed in this group.

The incidence of selected lesions is summarized from the report as follows:

Observation	Dose group * Control	Low	Mid	High
	F_2 Generation			
Litter A				
# Pups examined	37	43	-	43
# Non-fused supraoccipital bones	0	0	-	0
# Caudal vertebrae/pup*	8.0 (3-12)	7.2 (4-11)	-	6.8 (3-12)
# Tarsal bones (unilateral)/group	28	26	-	28
# Metacarpals + phalanges per foreleg*	14.1(13-17)	13.2(12-15)	-	13.3(13-16)
# Metacarpals + phalanges per hind leg*	14.0(13-14)	13.8(10-14)	-	13.8(9-14)

* Mean (range of observations)

Mean = $\frac{\text{Sum of bones ossified per group}}{\text{Number of pups per group}}$

ii. Microscopic Examination: Microscopic examinations were conducted on the liver, kidney, heart, spleen, adrenal, thyroid, gonads and bone marrow from 1 male and 1 female weanling of each of 12 - 13 litters per dose level from the F_{3b} generation. There were no treatment-related effects. The tables indicated that most of the tissues were within normal limits, except for the liver and kidney, which tended towards moderate congestion in all groups, including controls.

III. DISCUSSION

A. Investigators' conclusions: The investigators concluded that the only effects seen in the study were significantly lower ovary weights in the F_0 generation treated females. It is noted that the ovary weights were only measured in this generation. In addition, they stated that "some uncertainty must be attributed to 'stillborn' and 'died prior to weaning' numbers since these include dam-induced deaths through accident, mutilation, or maliciousness."

B. Reviewer's discussion: This study was conducted in 1969 and has some major deficiencies. The study is graded as Core Supplementary. In general, the information from a reproduction study needs to be sufficient to supply adequate data to predict parental toxicity, potential reproductive toxicity along with any potential effects on pups. The data in this study are insufficient to accurately predict these parameters. The following list discusses some of the deficiencies in the study.

Parental Toxicity Considerations:

1. Only two dose levels were tested. The EPA Testing Guidelines call for 3 dose levels to be tested, so that any dose responses may be determined.

2. The highest dose level did not induce toxicity. Therefore, it is uncertain as to whether or not the dams were tested at sufficiently high dose levels for a "negative" study.

3. The body weight data were presented across the entire duration of each generation. In order to assess whether or not an effect was observed on body weights, they need to be measured and presented separately for the pre-mating periods, the gestation and the lactation periods. As the data are presented in this study, one can only estimate where the mating periods began. In addition, the body weights were not measured for dams during the gestation and lactation periods. Therefore, from the data, one can estimate that there appeared to be no effects during the pre-mating periods, but since the bodyweights were not measured during gestation and lactation, one cannot determine whether or not there were effects during these times.

4. There were no summary tables or individual animal data for clinical signs observed or for macroscopic examinations. If there were absolutely no clinical signs or gross observations, it should have been stated somewhere as to why no tables were available in the report.

5. Microscopic examinations were not conducted on the reproductive organs of the parental animals. These examinations need to be done on at least the control and high dose groups.

Reproductive Toxicity Considerations:

1. There were between 14 - 22 litters available for examination per dose level at any one time during the study. Most of the time, less than 20 litters were available. The Guidelines state that for statistical considerations, at least 20 litters per dose level be available for examination.

2. From the available data, the number of dams that mated (usually determined by the presence of vaginal plugs) or the number of dams that were pregnant for each subgeneration (i.e. F_{1a} versus F_{1b}) could not be estimated. From the implantation data, the number of

females that had been pregnant could be estimated, but only for the entire generation (2 litters). Since these data were not available, the mating (# mated/# paired), fertility (# pregnant/# mated) and the gestation indices (# live litters/# pregnant) for each subgeneration could not be estimated.

3. The pups were not sexed except in the limited visceral examinations that were conducted. Therefore, an examination of the sex ratio could not be conducted.

4. The precoital and the gestation intervals were not measured.

Pup Toxicity Considerations:

Macroscopic examinations were conducted on the pups but were not reported.

General Considerations:

There were no analyses for material stability, homogeneity and concentration in the dosing medium.