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Reviewer: Whang Phang, Ph.D. 11/28/89
HFAS/Tox.Branch II (H7509C)

Secondary Reviewers: Steve Dapson, Ph.D. 11/30/89
Clark Swentzel, Section Head 11/30/89
HFAS/Tox. Branch II (H7509C)

ADDENDUM TO DATA EVALUATION REPORT OF A TWO-GENERATION REPRODUCTION STUDY ON DEET

Chemical: Deet Caswell No. 346

EPA MRID No.: 409790-01 HED Project No. 9-1303

Citation: Schardein, JL, Evaluation of deet in a two-generation reproduction/fertility study in rats. International Research and Development Corp.; Study No.: 555-004; Jan. 23, 1989. (Submitted by Deet Joint Venture/ Chemical Specialties Manufacturers Association)

Discussion: This study was evaluated and a data evaluation record (DER) was prepared by Dynamac Corp.. However, the HFAS/Tox. Branch II does not agree with certain scientific judgments made by Dynamac, and the Branch has recommended appropriate changes be made. Dynamac felt strongly about their decisions, and the suggested changes were not made. Besides the following disagreements, all other aspects of the DER are acceptable to the Branch.

1). Food consumption (pages and 8 & 15 of the DER): The Dynamac reviewer identified that there was a decrease in food consumption in F_0 males of mid- and high-dose groups and in F_1 males of the high dose group. However, the decrease was small, and it could not be considered as biologically significant (see Table 2, page 11 of the DER).

2). Reproductive toxicity (pages 10 & 15 of the DER): There was a statistically significant decrease in body weight of high-dose F_1 and F_2 males on lactation days 7, 14, and 21. A similar decrease was also seen in high dose F_1 and F_2 females on days 14 and 21. The Dynamac reviewer considers this decrease in body weight as developmental toxicity (pages 4 & 16 of the DER). HFAS/Tox. Branch II does not agree with this interpretation because the food intake for lactating rats is substantially higher during this period of growth relative to the mature rats. Consequently, these rats received a greater amount of the test chemical. Therefore, it would be more appropriate to consider the decrease in body weight as a systemic effect than a developmental effect.

3). Study deficiencies: There were 3 deficiencies indentified

by the Dynamac reviewer, but these deficiencies did influence the interpretation of the results.

Conclusion: Based upon the results presented in the study, the NOEL for parental toxicity could not be established, and the LOEL was 500 ppm (lowest tested dose produced kidney effects which included mottling, inflammation, presence of hyaline droplets, granular cast formation, and tubular regeneration).

No reproductive or developmental toxicity was found, the NOEL for these two parameters was 5000 ppm (highest tested dose).

The study is classified as core minimum.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68D80056
DYNAMAC No.: 204-A
TASK No.: 2-04A
November 15, 1989

DATA EVALUATION RECORD

DEET

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Schardein, J. L. Evaluation of deet in a two-generation reproduction/fertility study in rats. (Unpublished study No. 555-004 conducted by International Research and Development Corporation, Mattawan, MI, and submitted by Deet Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC; dated January 23, 1989.) MRID No. 409790-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 11/10/89

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EPA: 68D80056
DYNAMAC No.: 204-A
TASK No.: 2-04A
November 15, 1989

DATA EVALUATION RECORD

DEET

Two-Generation Reproduction Study in Rats

REVIEWED BY:

Patricia A. Turck, M.S.
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Date: November 15, 1989

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DATA EVALUATION RECORD

STUDY TYPE: Reproductive toxicity: Guideline §83-4.

MRID NUMBER: 409790-01.

TEST MATERIAL: Deet.

SYNONYM: N,N-diethyl-m-toluamide.

STUDY NUMBER: 555-004.

SPONSOR: Deet Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC.

TESTING FACILITY: International Research and Development Corporation, Mattawan, MI.

TITLE OF REPORT: Evaluation of Deet in a Two-Generation Reproduction/Fertility Study in Rats.

AUTHOR: Schardein, J. L..

REPORT ISSUED: January 23, 1989

5

CONCLUSIONS:

In a reproductive toxicity study in which groups of Sprague-Dawley rats were fed diets containing 0, 500, 2000, or 5000 ppm of deet for two consecutive generations, kidney effects were noted in all treated males. Therefore, LOEL for parental toxicity was 500 ppm; the NOEL was not established.

No compound-related effects on reproductive parameters, i.e., fertility, gestation, and viability, were observed. Developmental toxicity as evidenced by significant reductions in the body weights of pups from the high-dose group beginning at day 7 of lactation for males and day 14 of lactation for females was observed. The same pattern of reduced body weights occcurred over two generations. Based on the results of this reproductive toxicity study, the NOEL and LOEL were 2000 and 5000 ppm, respectively.

Classification: CORE Minimum.

IMPURITY INFORMATION IS NOT INCLUDED

A. MATERIALS:

Test Compound: Purity: 100%.
Description: Pale yellow liquid.
Lot No.: A-1-96.
Contaminants: [redacted]

Vehicle(s): No vehicle was used; the test material was mixed directly with feed.

Test Animals: Species: Rat.
Strain: Sprague-Dawley (COBS CD).
Source: Charles River Laboratories, Portage, MI.
Age: Animals were 42 days of age at initiation of the study.
Weight: Females, 115-171 g; Males, 166-219 at initiation of the study.

B. STUDY DESIGN:

This study was designed to assess the reproductive toxicity potential of deet when administered in the diet for two successive generations.

Mating: At sexual maturation and after at least 80 days on the test diets, parental animals from the same dietary group were paired 1:1. A maximum of 21 days was allowed for mating. However, if no evidence of mating was observed after 7 days of cohabitation, the female was placed with a proven male from the same dietary group. This was repeated, if necessary, in another 7 days. The day on which a vaginal plug was found was designated as day 0 of gestation. Parental animals produced only one litter. After weaning (day 21 of lactation) and acclimation to test diets for 1 week, 28 males and 28 females were randomly chosen to serve as parents for the next generation and were individually housed. After at least 93 days on the test diets, the animals were mated as described above. Sibling pairings were avoided.

Group Arrangement: The animals were randomly allocated to test groups using a computer-generated, weight-stratified randomization procedure.

Test group	Dietary Concentration (ppm)	Number assigned per group	
		Males	Females
Control	0	28	28
Low dose	500	28	28
Mid dose	2000	28	28
High dose	5000	28	28

Dosing: The test material was administered in the diet for two consecutive generations. Diets were prepared weekly and analyzed for concentration during weeks 1 to 4 and monthly thereafter. Homogeneity and stability analyses were conducted prior to study initiation. The test material was stable at room temperature for 10 days and was homogeneous after blending for 20 minutes (this was adopted for diet preparation during the study). Dietary levels were chosen based on a preliminary 90-day range-finding study. No other information on the preliminary study was provided.

Observations: The animals were observed twice daily for mortality and overt signs of toxicity. In addition, a detailed clinical observation was performed once a week. During this observation, findings as well as site, location, and description of palpable masses were recorded.

The body weights of males and females (until gestation) were recorded weekly. During gestation, dams were weighed on days 0, 6, 15, and 20; during lactation, dams were weighed on days 0, 7, 14, and 21.

Food consumption was recorded weekly, except for the mating period for males and the mating, gestational, and lactational periods for females. During gestation and lactation, the food consumption of dams was measured on days 0-7, 7-14, and 14-21.

The day on which all pups from a litter were delivered was designated as day 0 of lactation. The following data were recorded for each litter:

- Number of live and stillborn pups per litter at birth;
- External anomalies;
- Sex and body weight by sex on days 0, 4, 7, and 14 of lactation;
- Sex and individual body weights on day 21 of lactation; and
- Survival and behavioral abnormalities at least twice daily during the lactational period.

Litters were randomly culled to eight pups (four/sex, if possible) on day 4 of lactation. Pups culled or dying during the lactational period were examined for external abnormalities and were necropsied to determine the cause of death. Any tissues with lesions were preserved in neutral-buffered formalin (10%) for possible histopathological examination. After weaning, 10 pups/sex/group were subjected to a gross necropsy. Particular attention was given to reproductive organs (see below for further description of necropsy procedures). Following selection of parental animals, the remaining pups were examined externally and discarded.

Parental F_0 females were sacrificed after the selection of F_1 parents; F_1 females were sacrificed after weaning of their litters. All parental animals and 10 weanlings/sex/group were subjected to a complete necropsy, during which contents of the cranial, thoracic, and abdominal cavities were examined. The following organs were collected and fixed in phosphate-buffered neutral formalin:

- ovaries
- prostate
- seminal vesicles
- testes with epididymis
- uterus
- vagina
- all gross lesions.

The above tissues were microscopically examined for all control and high-dose parental animals. Tissues from the remaining animals, including weanlings, were saved for possible histopathological evaluation.

Statistical Analysis:

- Parental body weight, pup body weight, food consumption, and number of liveborn pups/litter--Bartlett's homogeneity test and ANOVA followed by Dunnett's test for multiple comparisons.
- Fertility indices--Chi-square test with Yates' correction factor and/or Fisher's exact test.
- Pup survival indices--Mann-Whitney U-test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated January 17, 1989, was provided.
- A signed Statement of Compliance with EPA GLPs (not dated) was provided.
- A signed Quality Assurance Statement, dated January 23, 1989, was provided.

C. RESULTS:

1. Dose Analysis: Analysis indicated that actual dietary concentrations ranged from 90 to 108% of target concentrations.

2. Parental Toxicity:

Mortality: One F_0 female from the control group died during the study as a result of mechanical injuries. One F_0 female from the high-dose group was sacrificed in extremis during parturition because of dystocia. One F0 male from the mid-dose group was sacrificed in extremis as a result of mechanical injury. No other deaths were reported.

Clinical Observations: Hair loss in the F_0 and F_1 females appeared to be slightly higher at the high-dose level when compared to controls. No other differences in the incidence of any clinical observations were observed among control and test groups.

Body Weight: A summary of body weight data are presented in Table 1. Body weights beginning at 7 and 8 weeks of age of F_0 males from the mid- and high-dose groups and F_1 males from the high-dose group, respectively, were consistently below controls (significant at $p<0.05$ to 0.01) through the entire treatment period, except for F_0 males in the high-dose group during weeks 20 to 24.

The body weights of F_0 and F_1 females were reduced during the entire treatment period. In F_0 females, a slight reduction was observed for the high-dose group during the first 9 weeks on study; significant reductions ($p<0.05$) were observed during the 2 weeks preceding pairing and the 4 weeks after lactation was completed. During the F_1 generation, high-dose females were significantly lighter ($p<0.01$ to 0.05) throughout the entire premating period.

Maternal weight gain during gestation and lactation was similar among control and test groups for the F_0 generation (data not shown). During the F_1 generation, significant increases ($p<0.05$) in weight gain during gestational days 6 to 15 were observed in mid- and high-dose dams. During lactation, a significant increase in weight gain ($p<0.05$) during days 14 to 21 was noted in high-dose dams.

Food Consumption: The food consumption of F_0 males from the mid- and high-dose groups was consistently lower and frequently significantly lower than controls throughout the premating period. In addition, the food consumption values for low-dose F_0 males was slightly lower than that of controls; sporadic but significant reductions were observed. During the F_1 generation, consistent reductions (frequently significant) during the premating period were again observed for high-dose males when compared with controls. However, food consumption was similar among the control, low-dose, and mid-dose F_1 males.

During the F_0 generation, food consumption values were slightly lower but sporadically significant during the premating period for high-dose females. However, the g/food consumed/kg of body weight for high-dose females was generally comparable to or greater than that of controls (data not presented). During premating period of

TABLE 1. Summary of Body Weight Data for Rats Fed Deet for Two Generations[a]

Dietary concentration (ppm)	Mean body weight (g ± S.D.) at week (age): 6	12	18	24	25
F_0 males					
0	197 ± 12.7	436 ± 35.1	520 ± 52.8	582 ± 67.0	--[b]
500	193 ± 13.1	426 ± 32.6	503 ± 41.0	578 ± 53.6	--
2000	190 ± 11.9	410 ± 28.7**	486 ± 38.4*	546 ± 49.5*	--
5000	191 ± 10.6	410 ± 26.6**	484 ± 37.2**	553 ± 47.1	--
F_0 females					
0	142 ± 10.4	223 ± 26.6	269 ± 28.7[c]	--[d]	282 ± 30.7
500	147 ± 9.2	231 ± 19.0	257 ± 25.1	--	284 ± 23.3
2000	149 ± 10.5*	229 ± 26.5	255 ± 31.1	--	280 ± 28.2
5000	143 ± 11.3	216 ± 15.2	234 ± 15.1*	--	265 ± 13.8*
F_1 males					
0	195 ± 18.3	439 ± 35.9	524 ± 52.5	597 ± 60.8	--[b]
500	194 ± 14.8	428 ± 36.7	507 ± 48.6	573 ± 64.4	--
2000	192 ± 17.7	417 ± 47.3	506 ± 54.6	584 ± 65.0	--
5000	186 ± 14.1	405 ± 26.9**	480 ± 36.4**	543 ± 42.7**	--
F_1 females					
0	147 ± 10.7	244 ± 24.4	293 ± 35.2	--[d]	--
500	147 ± 9.9	246 ± 18.3	292 ± 26.9	--	--
2000	147 ± 9.5	239 ± 18.5	274 ± 25.1	--	--
5000	139 ± 11.7*	221 ± 20.3**	248 ± 20.3**	--	--

[a]Data were extracted from study No. 555-004, Tables 5, 6, 8, and 9 and Appendix B.

[b]F_1 males were sacrificed at 25 weeks of age; F_1 males were sacrificed at 27 weeks of age.

[c]Values represent body weights at 17 weeks of age.

[d]Mating, gestation, or lactation occurred during this period.

*Significantly different from controls ($p<0.05$).

**Significantly different from controls ($p<0.01$).

the F_1 generation, consistent, significant reductions ($p<0.01$ for the majority of weekly intervals) in food consumption were observed in high-dose females. For mid-dose females, slight but sporadically significant reductions were also observed. Food consumption during the premating period was comparable between controls and low-dose females.

No differences in food consumption during the gestational and lactational periods were observed among control and test groups during the F_0 generation. During the F_1 generation, food consumption was significantly reduced during days 7 to 14 of gestation ($p<0.05$) and days 0 to 7 of lactation ($p<0.01$) for high-dose females. A compensatory increase ($p<0.05$) in food consumption during days 14 to 21 of gestation was noted in high-dose F_1 females. During days 0 to 7 of lactation, food consumption was significantly reduced ($p<0.05$) in low-dose females when compared to controls. However, food consumption during all other gestational and lactational intervals was omparable between control and low-dose females.

A summary of food consumption data collected during the premating interval is presented in Table 2.

Gross and Microscopic Pathology: A dose-related increase in the incidence of mottled kidneys was observed in F_1 males; this lesion was observed in 0, 2 (9%), 6 (26%), and 8 (35%) males from the control and low-, mid-, and high-dose groups, respectively. Microscopic observation revealed inflammation, hyaline droplet and granular cast formation, congestion, and regeneration of tubules in the kidneys of these animals. These findings were associated with alpha 2u globulin nephropathy.

3. Reproductive Toxicity: A summary of effects on reproductive parameters and offspring survival for the F_0 and F_1 generations are presented in Tables 3 and 4, respectively. The only effect noted was a significant decrease ($p<0.05$) in the viability of F_2 low- and high-dose pups during the first 4 days of lactation.

Birth weights among control and test groups were similar (Table 5). However, reductions in body weight of high-dose pups were observed as lactation progressed. Significant decreases ($p<0.05$ to 0.01) in body weight were observed on days 7, 14, and 21 in high-dose F_1 and F_2 males and on days 14 and 21 in high-dose F_1 and F_2 females when compared to controls. In addition, the body weight of mid-dose F_2 females was slightly below that of controls, but the difference was not statistically significant.

One and two F_2 pups culled on day 4 of lactation, as well as two (culled) and three F_2 pups (two died and one was culled; from two related dams) from control and high-dose

12

TABLE 2. Summary of Food Consumption (g/animal/day) for Rats Fed Deet for Two Generations[a]

Dietary concentration (ppm)	Mean food consumption (g ± S.D.) at week (of age): 7	9	12	16
F_0 males				
0	24 ± 1.8[b]	25 ± 2.4	25 ± 2.3	26 ± 2.8
500	23 ± 1.9*	24 ± 2.3	25 ± 2.1	26 ± 3.1
2000	22 ± 1.3**	24 ± 1.7	24 ± 1.6*	25 ± 2.4
5000	22 ± 1.4**	23 ± 1.7**	24 ± 1.6	25 ± 2.2
F_0 females				
0	14 ± 3.7	16 ± 1.9	16 ± 2.5	17 ± 2.0
500	16 ± 0.9*	16 ± 1.4	17 ± 1.6	18 ± 1.9
2000	15 ± 1.6	16 ± 1.9	17 ± 2.7	18 ± 2.6
5000	15 ± 2.7	15 ± 1.1**	16 ± 1.4	16 ± 4.0
F_1 males				
0	25 ± 2.0[c]	30 ± 2.6	29 ± 2.5	29 ± 2.7
500	25 ± 5.5	29 ± 5.8	28 ± 3.5	29 ± 2.2
2000	24 ± 2.0	28 ± 2.8	28 ± 3.6	30 ± 4.0
5000	23 ± 1.7*	28 ± 1.8**	27 ± 2.7	28 ± 3.3
F_1 females				
0	19 ± 1.4[c]	20 ± 3.5	20 ± 2.3	21 ± 2.6
500	20 ± 2.7	20 ± 2.3	21 ± 3.4	21 ± 3.3
2000	19 ± 1.3	18 ± 3.6	19 ± 1.5**	20 ± 2.7
5000	18 ± 1.4**	18 ± 1.6*	17 ± 1.6**	18 ± 2.0**

[a]Data were extracted from study No. 555-004, Tables 11, 12, 14, and 15 and Appendix B.

[b]Mean ± S.D.

[c]F_1 animals were 6 weeks of age.

*Significantly different from controls ($p<0.05$).

**Significantly different from controls ($p<0.01$).

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TABLE 3. Summary of Effects of the Dietary Administration of Deet on F_0 Reproductive Parameters and Offspring Survival[a]

Parameter	Dietary concentration (ppm) 0	500	2000	5000
No. matings	28	28	28	28
No. pregnancies/females	23	21	26	28
Fertility index (%)	82.1	75.0	92.9	100.0
Gestation index (%)[b]	100.0	100.0	100.0	100.0
Mean gestation length (days)	21.9	21.9	22.0	22.0
Total No. pups born	267	256	338	345
Mean litter size at birth	11.6 ± 2.23[c]	12.2 ± 2.11	13.0 ± 2.26	12.3 ± 2.65
Total No. pups alive, day 0	263	253	330	335
Mean No. live pups/litter[b]	11.4 ± 2.33	12.0 ± 2.13	12.7 ± 2.33	12.0 ± 2.92
Live birth index (%)[d]	98.5	98.8	97.6	97.1
Total No. pups alive, day 4	248	246	325	322
Mean No. live pups/litter[b]	11.3 ± 2.31	11.7 ± 2.19	12.5 ± 2.23	11.5 ± 3.06
Viability index 1 (%)[e]	98.8	97.2	98.5	96.1
Total No. pups alive, day 21	173	168	206	209
Mean No. live pups/litter[b]	7.9 ± 0.47	8.0 ± 0.0	7.9 ± 0.27	7.7 ± 0.71
Viability index 2 (%)[f]	100.0	100.0	100.0	99.0

[a]Data were extracted from study No. 555-004, Tables 20 and 21 and Appendix B.

[b]Calculated by the reviewers using individual animal data.

[c]Mean ± S.D.

[d]Live birth index = $\frac{\text{No. pups alive on day 0}}{\text{No. pups born alive}} \times 100$.

[e]Viability index 1 = $\frac{\text{No. pups alive on day 4 before culling}}{\text{No. pups born alive}} \times 100$.

[f]Viability index 2 = $\frac{\text{No. pups alive on day 21}}{\text{No. pups alive on day 4 after culling}} \times 100$; calculated by the reviewers.

TABLE 4. Summary of Effects of the Dietary Administration of Deet on F_1 Reproductive Parameters and Offspring Survival[a]

Parameter	Dietary concentration (ppm) 0	500	2000	5000
No. matings	28	28	28	28
No. pregnancies	21	25	25	25
Fertility index/female (%)	75.0	89.3	89.3	89.3
Gestation index (%)[b]	100.0	88.0	100.0	100.0
Mean gestation length (days)	21.9	22.1	21.9	22.0
Total No. pups born	275	275	322	350
Mean litter size at birth	13.1 ± 1.97[c]	12.7 ± 3.45	12.9 ± 3.57	14.0 ± 2.06
Total No. pups alive, day 0	271	272	320	344
Mean No. live pups/litter[b]	12.9 ± 2.07	12.4 ± 3.36	12.8 ± 3.53	13.8 ± 2.18
Live birth index (%)[d]	98.5	98.9	99.3	98.3
Total No. pups alive, day 4	271	268	318	335
Mean No. live pups/litter[b]	12.9 ± 2.07	12.2 ± 3.32	12.7 ± 3.63	13.4 ± 2.20
Viability index 1 (%)[e]	100.0	98.5*	99.3	97.4*
Total No. pups alive, day 21	167	168	189	199
Mean No. live pups/litter[b]	7.9 ± 0.22	7.6 ± 1.33	7.6 ± 1.53	8.0 ± 0.20
Viability index 2 (%)[f]	100.0	100.0	100.0	99.5

[a]Data were extracted from study No. 555-004, Tables 20 and 21 and Appendix B.

[b]Calculated by the reviewers using individual animal data.

[c]Mean ± S.D.

[d]Live birth index = $\frac{\text{No. pups alive on day 1}}{\text{No. pups born alive}} \times 100$.

[e]Viability index 1 = $\frac{\text{No. pups alive on day 4 before culling}}{\text{No. pups born alive}} \times 100$

[f]Viability index 2 = $\frac{\text{No. pups alive on day 21}}{\text{No. pups alive on day 4 after culling}} \times 100$; calculated by the reviewers.

*Significantly different from controls ($p<0.05$).

15

TABLE 5. Summary of Body Weight Data in the Offspring of Rats Fed Deet for Two Generations[a]

Dietary concentration (ppm)	Mean body weight (g ± S.D.) on lactational day: 0	7	14	21
F_1 male pups				
0	6.0 ± 0.70	15.1 ± 1.87	29.6 ± 3.42	46.2 ± 6.50
500	6.3 ± 0.62	14.9 ± 1.67	28.7 ± 3.28	46.8 ± 5.06
2000	6.2 ± 0.61	15.3 ± 1.65	28.6 ± 2.45	45.7 ± 4.53
5000	6.2 ± 0.70	13.9 ± 1.68*	25.8 ± 3.56**	40.1 ± 4.22**
F_1 female pups				
0	5.8 ± 0.67	14.4 ± 1.79	28.8 ± 2.69	44.1 ± 4.64
500	5.9 ± 0.47	14.1 ± 1.86	28.5 ± 3.66	44.6 ± 4.44
2000	6.1 ± 1.00	14.8 ± 1.86	27.6 ± 2.09	44.2 ± 4.51
5000	6.1 ± 0.74	13.8 ± 2.54	25.5 ± 3.78**	39.1 ± 4.60**
F_2 male pups				
0	6.3 ± 0.80	16.0 ± 1.40	32.7 ± 2.31	50.4 ± 4.31
500	6.3 ± 0.58	15.7 ± 2.26	31.7 ± 3.44	49.4 ± 6.07
2000	6.2 ± 0.53	15.7 ± 1.27	31.5 ± 3.43	47.9 ± 4.02
5000	6.2 ± 0.45	14.9 ± 1.20**	29.1 ± 2.19**	44.5 ± 3.93**
F_2 female pups				
0	5.7 ± 0.40	15.1 ± 1.54	31.0 ± 2.51	47.3 ± 4.52
500	6.1 ± 0.59	15.5 ± 1.73	30.9 ± 2.69	47.6 ± 5.35
2000	5.9 ± 0.63	14.7 ± 1.69	29.5 ± 4.79	44.1 ± 7.50
5000	5.9 ± 0.41	14.3 ± 1.17	28.1 ± 1.95**	42.3 ± 3.23**

[a]Data were extracted from study No. 555-004, Tables 20 and 21 and Appendix B.

*Significantly different from controls ($p<0.05$)

**Significantly different from controls ($p<0.01$)

groups, respectively, had heart and large vessel malformations. One F_2 pup from the high-dose group had microphthalmia. One F_2 pup from the control group had retroesophageal subclavian (right).

D. DISCUSSION/CONCLUSION:

1. Parental Toxicity: The body weights of F_0 males from the mid-and high-dose groups and of F_1 males from high-dose group were consistently lower than control values. These decreases were supported by reductions in food consumption in F_0 males from the mid- and high-dose groups and F_1 males from the high-dose group. However, calculation of normalized food consumption (g/kg/day) revealed significant reductions in high-dose F_0 males only during weeks 1 to 3 and 8 of treatment, whereas food consumption for high-dose F_1 males was similar to that of controls throughout the treatment period. Kidney effects that included mottled kidneys, as well as inflammation, hyaline droplet and granular cast formation, and tubular regeneration were observed in F_1 males from all test groups. These lesions were not observed in F_0 males. However, only gross lesions were histologically examined, and mottling of the kidneys was not observed in F_0 males.

Females were not as severely affected as males; consistent reductions in body weight were observed at the high-dose level only. Body weight was significantly reduced in F_0 females beginning at week 10 of treatment (16 weeks of age) and in F_1 females throughout the entire treatment period. No kidney effects were observed in females.

The study author associated the kidney effects observed in F_1 males with alpha 2u globulin nephropathy, a chemically induced phenomenon that has been seen only in male rats. However, this was based on morphological evidence alone; the presence of alpha 2u globulin was not determined by chemical analysis. Furthermore, although this phenomenon has been seen only in male rats, the significance of this finding for the human population is not known. Therefore, as a conservative measure, we assess that the kidney effects observed in F_1 males from all test groups are a toxic effect.

The parental LOEL was 500 ppm; the parental NOEL was not established.

2. Reproductive Toxicity: A slight reduction in fertility was observed during the F_0 generation in low-dose females. Fertility for mid- and high-dose F_0 females, however, was greater than that of controls; therefore, this was not considered to be compound related. Reduced viability among low- and high-dose F_2 pups was noted during days 0 to 4 of lactation. Although significantly different from controls, it was within the range of historical controls. The body weights of male and female pups in the high-dose group were significantly reduced from day 7 for males and day 14 for females. Because this effect was observed for two consecutive generations and body weight reduction in neonates may have secondary effects on the development of various functions, the weight reduction should be considered when determining the NOEL and LOEL for the second and third generation pups. The malformations observed in the heart and large vessels appeared to be a genetic flaw and were not considered to be compound related.

Based on the developmental effects noted at the high-dose level, the NOEL was 2000 ppm, and the LOEL was 5000 ppm.

3. Study Deficiencies: The following deficiencies were found:

1. No data on concentration analysis other than percent of target was reported; therefore, summary data could not be verified.

2. Individual data on pups such as body weights and necropsy findings of culled pups or pups dying during the study were not presented. Therefore, summary tables could not be verified.

3. Although the kidney was identified as a target organ, the kidneys from all animals, particularly F_1 and F_2 weanlings, were not examined histologically.

E. CLASSIFICATION: CORE Minimum.

Parental NOEL = Not established.
Parental LOEL = 500 ppm.
Reproductive toxicity NOEL = 2000 ppm.
Reproductive toxicity LOEL = 5000 ppm.

F. RISK ASSESSMENT: Not applicable.