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

MAR 17 1992

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Two-generation Reproduction Toxicity in rats with AC 6601 (Malathion).

Tox.Chem No.: 535
MRID No.: 415834-01
HED Project No.: 0248
Submission No.: S383545

To: Joanne Edwards
PM Team# 74
SR Branch
SRRD (H7508W)

From: Henry Spencer, Ph.D. 3/10/92
Acting Section Head Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

Thru: Karl Baetcke, Ph.D. 3/12/92
Chief
Toxicology Branch 1
Health Effects Division (H7509C)

ACTION:

Review a Two-generation reproduction study (Guidelines 83-4) in rats exposed to Malathion. The study package contained five volumes of data (study No. 87-3243) from BioDynamics sponsored by American Cyanamid Co. dated June 28, 1990.

CONCLUSIONS:

In the Two-generation (2 litters) study in rats given diets of 0, 550, 1700, 5,000 or 7,500 ppm of AC 6601, toxicity to the dams was seen as reduced body weight in F_0 females during gestation and lactation at 7500 ppm as well as the F_1 males and females during the pre-mating period. The NOEL was 5,000 ppm (394 mg/kg for males, 451.4 mg/kg for females) with an LEL equal to 7500 ppm (corresponding to 611.5 mg/kg for males and 703 mg/kg for females) for parental toxicity. Reduced body weight (pups, growth) during lactation was demonstrated. A NOEL can be demonstrated at 1700 ppm

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(NOEL 153.1 mg/kg), with 5000 ppm as an LEL (451.4 mg/kg) for reproductive toxicity.

The study satisfies the 83-4 guideline requirements. Core classified as: minimum data.

CONFIDENTIAL BUSINESS INFORMATION
DOES N... ...
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68D80056
DYNAMAC No.: 341-A
TASK No.: 3-41A
February 1, 1991

DATA EVALUATION RECORD

AC 6,601

Two-Generation Reproductive Toxicity Study in Rats

STUDY IDENTIFICATION: Schroeder, R.E. A two-generation (two litters) reproduction study with AC 6,601 to rats. (Unpublished study No. 87-3243 conducted by Bio/dynamics, Inc., East Millstone, NJ, and submitted by American Cyanamid Company, Princeton, NJ; dated June 28, 1990.) MRID No. 415834-01.

APPROVED BY:

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

Signature: [signature]
Date: 1/31/91

1. CHEMICAL: S-[1,2-bis(ethoxycarbonyl)ethyl]O,O-dimethylphosphorodithioate.

2. TEST MATERIAL: AC 6,601, lot No. AC 6015-136, 94.0% pure (prior to study initiation), clear, brown to colorless liquid.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Schroeder, R.E. A two-generation (two litters) reproduction study with AC 6,601 to rats. (Unpublished study No. 87-3243 conducted by Bio/dynamics, Inc., East Millstone, NJ, and submitted by American Cyanamid Company, Princeton, NJ; dated June 28, 1990.) MRID No. 415834-01.

5. REVIEWED BY:

Pia Lindström, DPH Principal Reviewer Dynamac Corporation	Signature: [signature] Date: 1-31-91
Patricia Turck, M.S. Independent Reviewer Dynamac Corporation	Signature: [signature] Date: February 1, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D. Department Manager Dynamac Corporation	Signature: ____ Date: ____
Brian Dementi, Ph.D. EPA Reviewer Review Section I Toxicology Branch I (H-7509C)	Signature: ____ Date: ____
Roger Gardner, Ph.D. EPA Section Head Review Section I Toxicology Branch I (H-7509C)	Signature: [signature] Date: [illegible]

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DATA EVALUATION RECORD

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

MRID NUMBER: 415834-01.

TEST MATERIAL: AC 6,601.

SYNONYMS: Malathion, mercaptothion, carbofos, maldison, Cythion, Calmathion, Detmol, MA 96%, Emmatos, For-Mal, Karbofos, Kop-Thion, Kypfos, Malaspray, Malamar, Malaphele, Prentox, Sumitox, Zithiol.

STUDY NUMBER: 87-3243.

SPONSOR: American Cyanamid Company, Princeton, NJ.

TESTING FACILITY: Bio/dynamics, Inc., East Millstone, NJ.

TITLE OF REPORT: A Two-Generation (Two Litters) Reproduction Study with AC 6,601 to Rats.

AUTHOR: Schroeder, R. E.

REPORT ISSUED: June 28, 1990.

CONCLUSIONS:

In a two-generation reproduction study in which COBS CD rats were fed diets containing AC 6,601 at 0, 550, 1700, 5000, and 7500 ppm (corresponding values for males, 0, 42.7, 130.9, 394.0, and 611.5 mg/kg/day, and for females 0, 50.6, 153.1, 451.4, and 703.4 mg/kg/day), parental toxicity, evidenced by reduced body weight, was observed in the F_0 females during gestation and lactation at 7500 ppm and in the F_1 males and females during the premating period. Based on these results, the NOEL and LOEL for parental toxicity were 5000 and 7500 ppm, respectively.

Fertility, length of gestation, and pup viability were unaffected by ingestion of the test material in the diet. However, pup growth (reduced body weight during lactation) was adversely affected at 5000 and 7500 ppm. Therefore, the NOEL and LOEL for reproductive toxicity were 1700 and 5000 ppm, respectively.

Classification: CORE Minimum Data. This study meets the minimum requirements set forth under Guideline 83-4 for a two-generation reproductive toxicity study in rats.

A. MATERIALS:

Test Compound:
- Purity: 94.0% prior to study initiation.
- Description: Clear, brown to colorless liquid.
- Lot No.: AC 6015-136.
- Contaminants: Not reported.

Vehicle: None used; the test material was administered in the diet.

Test Animals:
- Species: Rat.
- Strain: Sprague-Dawley (COBS CD).
- Source: Charles River Laboratories, Inc., Portage, MI.
- Age: Forty-two days at the start of the study.
- Weight: F_0 males--142-180 g, F_0 females--120-158 g and F_1 males--150-319 g and F_1 females--111-215 g at the start of exposure through the diet.

B. STUDY DESIGN:

This study was designed to assess the potential of AC 6,601 to cause reproductive toxicity, when administered continuously in the diet for two successive generations.

Mating: After 63 days of dietary treatment for F_0 parental animals, each male was cohabited with one female from the same group to produce the first litter. The day on which mating was confirmed by the presence of a copulatory plug or sperm in a vaginal smear was designated day 0 of gestation, and females were thereafter housed individually. Females not mated after 10 days of cohabitation were placed with previously proven breeder males for an additional 10-day period. Mating to produce the second litter was initiated following a 2-week resting period after weaning of the first litter. At approximately 100 days of age and 79 days of dietary treatment, F_1 parents (randomly selected from second F_0 litters) were mated in a similar fashion to achieve two litters. Sibling matings were avoided.

Group Arrangement: F_0 animals were randomly allocated to groups using a computerized sorting program based on body weight, and F_1 parental animals were randomly allocated to groups using a random numbers table as follows:

Test Group	Dietary Concentration (ppm)	Number Assigned per Group			
		F_0		F_1	
		Males	Females	Males	Females
I	0	25	25	25	25
II	550	25	25	25	25
III	1700	25	25	25	25
IV	5000	25	25	25	25
V	7500	25	25	25	25

Dosing: The test material was administered continuously in the diet for two consecutive generations. The test diets were prepared weekly and were adjusted for purity. The test compound was first diluted in acetone and then blended with several kilograms of Purina Rat Chow Certified diet #5002 in a Hobart-type mixing bowl to achieve a premix. The final concentrations were prepared by mixing the premix with appropriate amounts of rat chow in a blender. Stability (14 days) and homogeneity of the test material in the diet were determined for the low and high doses prior to study initiation. Purity of the test material was analyzed prior to study initiation and five times during the study. Concentration analyses of the test material in the diet were conducted on samples from each dose level taken weekly for the first 4 weeks and monthly thereafter. A rationale for the selection of doses was not reported.

Observations: Animals were observed twice daily for mortality, moribundity, and overt signs of toxicity. At least once weekly, animals were given a detailed physical examination. Body weights of males were recorded weekly throughout the study, and body weight of females were recorded weekly during the premating and resting periods, on gestational days (GD) 0, 7, 14, and 20, and on lactational days 0, 7, 14, and 21. Food consumption for males was recorded weekly throughout the study (except during mating) and for females weekly during the premating and resting periods and on GD 0-7, 7-14, and 14-20. During the mating and lactational periods, food consumption was not recorded for females.

The following data were recorded for each litter:

- Number of pups alive and dead at birth and on lactational days 4, 7, 14, and 21;

- Individual body weight and sex determination of live pups on lactational days 0, 4, 7, 14, and 21; and

- Mortality and general appearance twice daily.

On day 4 of lactation, all litters were culled to eight pups (four/sex if possible). Culled pups and pups found dead were weighed, sacrificed, given a gross external and internal examination (including internal sex determination), and preserved in 10% neutral buffered formalin. Each litter was weaned on day 21. F_{1A} pups were sacrificed after weaning. One week after weaning, one male and one female F_{1B} pup per litter were randomly selected as F_1 parental animals. In addition, one F_{1B} pup per sex per litter was selected for a gross examination between days 21 and 28, and gross lesions were preserved. The remaining F_{1B} pups were sacrificed on day 28 and subjected to a gross external examination. Pups with external abnormalities were examined internally. Gross lesions were preserved. The F_{2A} and F_{2B} pups were sacrificed on day 21 and evaluated in the same way as the F_1 pups.

Following weaning of the second litters and selection of the F_1 parental animals, the F_0 males and females were sacrificed and subjected to a gross examination. The following tissues were collected for possible histopathological examination:

- Vagina
- Uterus
- Ovaries
- Testes
- Epididymides
- Seminal vesicles
- Prostate
- Pituitary
- Gross lesion(s)

The F_1 parental males and females were sacrificed according to the same regimen following weaning and sacrifice of the F_2 litters. The tissues listed above from all F_0 and F_1 control and high-dose animals were examined microscopically. In addition, tissues that have been identified as targets of toxicity after examination of the high-dose group will be examined in all dose groups.

Statistical Analysis: The following analyses were conducted:

- Parental and pup body weight and parental body weight gain, food consumption, gestation length, and number of pups at birth--Bartlett's test for homogeneity, and ANOVA and Dunnett's test (parametric data) or Kruskal-Wallis and Dunn's test (nonparametric data);

- Mating, pregnancy, and fertility rates, pup and litter survival rates, and mortality rates--Chi-square analysis, Fisher's Exact test (with Bonferroni correction factor), and Armitage trend test;

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated August 2, 1990, was provided.

- A signed Statement of Compliance with EPA GLPs, dated June 28, 1990, was provided.

- A signed Quality Assurance Statement, dated April 5, 1990, was provided.

C. RESULTS:

The following results were reported by the study author.

1. Test Material Analysis: Purity of the test material ranged from 91.1 to 93.6% during the 2 years of refrigerated storage. Concentrations of the test material in the diets ranged from 85.0 to 115.2% of the nominal values. Homogeneity analyses revealed that samples were 106.7 and 97.7% of nominal values with coefficients of variation of 2.8 and 5.8% at 550 and 7500 ppm, respectively (excluding one sample reported as an outlier). Analyses for stability of the test material in the diet revealed the following results: After 7 days of storage at room temperature or frozen, samples from the low- and high-dose levels ranged from -4% to +7% of the concentration that had been determined on day 0. After 14 days of storage at room temperature or frozen, the low-dose concentrations were -11 and -12%, respectively, while the high-dose concentrations were -1 and -3%, respectively.

2. Parental Toxici...

Mortality: In the F_0 generation, one male from the 550-ppm group died on day 50 during the premating period. One female from the 5000-ppm group died 4 days after delivering the second litter (study day 170). Two females that died during the premating period (one from the control group that died on day 7 and the other from the 1700-ppm group that was killed accidentally on day 8) were replaced with two additional females. An additional male from the 550-ppm group was reported missing beginning on week 12.

In the F_1 generation, at 550 ppm, one male died just before the scheduled sacrifice (study day 191). Four females died, one each from the control (sacrificed moribund during the premating period on study day 67), 1700-ppm (died while delivering the second litter on study day 171), 5000-ppm (died during the second litter, postweaning period on study day 204), and 7500-ppm groups (died during the rest period on study day 143). No cause of death was reported for any of these animals.

Clinical Observations: The following observations were noted in both generations and sexes at similar frequencies in all dose groups: chromodacryorrhea, excess lacrimation, ear problems (red, encrusted, swollen, or torn), tooth problems (broken, cut, or maloccluded), and tail problems (not specified). Alopecia was noted in females from all groups in both generations. Single occurrences of the following were also observed: opacity, red nasal discharge, perforated palate, moist rales, sores, scabs, soft stool, and swollen snout. One high-dose F_1 male had a tissue mass (location not reported); however, there was no further discussion of the mass in the pathology report.

Body Weight: Summaries of body weights from selected time intervals are presented in Tables 1, 2, and 3.

In the F_0 generation, after the first mating, body weight in females was significantly ($p \leq 0.01$ or 0.05) decreased below controls at 7500 ppm on GD 7, 14, and 20 and during the entire lactational period and at 5000 ppm on lactational day 14. After the second mating, body weight was significantly ($p \leq 0.05$) decreased at 7500 ppm on lactational days 0 and 21.

In the F_1 generation, body weight was significantly ($p \leq 0.01$ or 0.05) decreased below controls at 7500 ppm during the entire treatment period for males and during premating weeks 1-3, 5, and 7-11 for females. Significant ($p \leq 0.05$) increases were noted in females at 1700 ppm on GD 14 and 20 during the first gestational period and at 1700 and 550 ppm on GD 14 and 20, respectively, during the second gestational period.

Food Consumption: Summaries of food consumption from selected time intervals are presented in Tables 4 and 5.

TABLE 1. Summary of Body Weights During the Premating Period for Rats Fed AC 6,601 for Two Successive Generations[a,b]

Dietary Concentration (ppm)	Mean Body Weight (g ± SD) at Study Week: 1	4	8	11
F_0 Males				
0	221 ± 13	349 ± 24	425 ± 38	---
550	223 ± 10	348 ± 17 (N=24)	435 ± 29 (N=23)	---
1700	222 ± 11	349 ± 19	429 ± 27	---
5000	220 ± 13	349 ± 19	431 ± 29	---
7500	213 ± 14	337 ± 20	417 ± 29	---
F_0 Females				
0	160 ± 10 (N=24)	213 ± 20	244 ± 24	---
550	158 ± 11	209 ± 20	238 ± 28	---
1700	158 ± 10	213 ± 18	249 ± 23	---
5000	159 ± 7	209 ± 14	244 ± 17	---
7500	158 ± 8	206 ± 12	239 ± 15	---
F_1 Males				
0	201 ± 38	336 ± 29	430 ± 32	483 ± 41
550	206 ± 28	333 ± 30	432 ± 37	487 ± 37
1700	211 ± 18	338 ± 18	436 ± 25	485 ± 27
5000	201 ± 18	320 ± 25	414 ± 43	464 ± 50
7500	174 ± 31**	299 ± 30**	391 ± 33**	435 ± 39**
F_1 Females				
0	149 ± 23	204 ± 25	243 ± 30	262 ± 38
550	151 ± 22	205 ± 25	247 ± 28	269 ± 32
1700	157 ± 11	212 ± 14	255 ± 16	277 ± 15
5000	147 ± 20	200 ± 17	237 ± 21	257 ± 25
7500	134 ± 24**	191 ± 17	226 ± 15*	244 ± 19*

[a]Data were extracted from study No. 87-3243, Tables 2 and 3.

[b]N=25 unless stated otherwise.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 2. Summary of Maternal Body Weights During Gestation in Rats Fed AC 6,601 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Body Weight (g ± S.D.) on Gestational Day: 0	7	14	20
F_0 generation - F_{1A} litter				
0	260 ± 22 (22)[b]	290 ± 24 (22)	319 ± 26 (22)	385 ± 32 (22)
550	254 ± 23 (22)	283 ± 25 (20)	313 ± 28 (22)	379 ± 33 (22)
1700	258 ± 22 (22)	287 ± 23 (22)	317 ± 24 (22)	382 ± 27 (22)
5000	253 ± 19 (24)	280 ± 21 (24)	312 ± 18 (24)	376 ± 22 (23)
7500	244 ± 16 (23)	268 ± 22* (23)	298 ± 24* (23)	358 ± 32* (23)
F_0 generation - F_{1B} litter				
0	302 ± 24 (19)	320 ± 23 (19)	352 ± 24 (19)	417 ± 26 (19)
550	287 ± 28 (19)	311 ± 30 (19)	343 ± 33 (19)	409 ± 39 (19)
1700	299 ± 26 (20)	323 ± 39 (20)	348 ± 40 (20)	413 ± 36 (20)
5000	288 ± 21 (19)	312 ± 23 (19)	342 ± 24 (19)	402 ± 28 (19)
7500	282 ± 16 (20)	304 ± 16 (20)	331 ± 17 (20)	396 ± 21 (20)
F_1 generation - F_{2A} litter				
0	254 ± 34 (20)	283 ± 34 (20)	310 ± 32 (20)	377 ± 39 (20)
550	268 ± 27 (22)	299 ± 31 (22)	326 ± 31 (22)	395 ± 38 (22)
1700	274 ± 16 (20)	306 ± 16 (20)	333 ± 18* (20)	407 ± 28* (20)
5000	253 ± 29 (23)	279 ± 31 (23)	310 ± 31 (23)	369 ± 44 (22)
7500	242 ± 19 (23)	261 ± 23 (23)	295 ± 23 (23)	356 ± 28 (23)
F_1 generation - F_{2B} litter				
0	292 ± 34 (16)	316 ± 32 (16)	337 ± 34 (16)	403 ± 45 (16)
550	312 ± 29 (20)	341 ± 33 (20)	362 ± 34 (20)	439 ± 38* (20)
1700	316 ± 17 (17)	344 ± 22 (17)	368 ± 26* (17)	438 ± 27 (17)
5000	293 ± 40 (14)	324 ± 45 (14)	351 ± 49 (14)	415 ± 57 (14)
7500	277 ± 23 (20)	300 ± 26 (20)	325 ± 28 (20)	391 ± 31 (20)

[a]Data were extracted from study No. 87-3243, Tables 26 and 27.

[b]Number of animals used in parentheses.

*Significantly different from controls ($p \leq 0.05$).

TABLE 3. Summary of Maternal Body Weights During Lactation in Rats Fed AC 6,601 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Body Weight (g ± S.D.) on Lactational Day: 0	7	14	21
F_1 generation - F_{1A} litter				
0	304 ± 31 (24)[b]	306 ± 26 (24)	321 ± 26 (24)	318 ± 24 (24)
550	294 ± 26 (23)	297 ± 20 (23)	305 ± 24 (23)	305 ± 33 (23)
1700	295 ± 22 (23)	298 ± 22 (22)	313 ± 21 (23)	321 ± 24 (23)
5000	291 ± 19 (24)	298 ± 15 (24)	302 ± 18* (24)	311 ± 21 (24)
7500	276 ± 19** (24)	285 ± 17* (24)	298 ± 18** (24)	297 ± 18* (24)
F_1 generation - F_{1B} litter				
0	330 ± 28 (24)	339 ± 24 (24)	345 ± 21 (24)	341 ± 22 (24)
550	322 ± 34 (22)	327 ± 33 (22)	332 ± 22 (21)	333 ± 27 (21)
1700	326 ± 36 (22)	337 ± 28 (23)	339 ± 27 (23)	340 ± 23 (23)
5000	318 ± 29 (21)	336 ± 22 (20)	340 ± 22 (20)	340 ± 22 (20)
7500	303 ± 20* (22)	325 ± 18 (22)	328 ± 16 (22)	320 ± 16* (22)
F_2 generation - F_{2A} litter				
0	293 ± 31 (24)	306 ± 29 (23)	309 ± 27 (23)	305 ± 25 (23)
550	300 ± 37 (25)	314 ± 30 (25)	313 ± 27 (25)	305 ± 25 (25)
1700	304 ± 25 (24)	317 ± 15 (23)	324 ± 16 (23)	320 ± 16 (23)
5000	287 ± 27 (23)	297 ± 28 (21)	301 ± 25 (22)	300 ± 22 (22)
7500	272 ± 23 (25)	288 ± 25 (25)	292 ± 19 (25)	292 ± 20 (25)
F_2 generation - F_{2B} litter				
0	323 ± 31 (17)	333 ± 31 (16)	334 ± 30 (16)	331 ± 25 (16)
550	345 ± 38 (20)	354 ± 33 (20)	347 ± 33 (20)	343 ± 34 (20)
1700	348 ± 30 (17)	359 ± 23 (16)	357 ± 20 (16)	355 ± 18 (16)
5000	319 ± 42 (15)	323 ± 29 (14)	333 ± 30 (14)	331 ± 33 (14)
7500	315 ± 29 (20)	328 ± 23 (20)	325 ± 23 (20)	324 ± 24 (20)

[a]Data were extracted from study No. 87-3243, Tables 32 and 33.

[b]Number of animals used in parentheses.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 4. Summary of Food Consumption During the Premating Period for Rats Fed AC 6,601 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Food Consumption (g/kg/day ± SD) at Study Week: 1	4	8	11
F0 Males				
0	122 ± 5.1 (25)[b]	79 ± 4.0 (25)	62 ± 4.6 (25)	---
550	122 ± 5.2 (25)	75 ± 4.5** (24)	59 ± 3.3 (23)	---
1700	121 ± 4.5 (25)	76 ± 3.3* (24)	59 ± 3.2* (25)	---
5000	123 ± 4.4 (23)	77 ± 3.7 (23)	60 ± 3.1 (25)	---
7500	119 ± 4.5 (20)	78 ± 3.9 (24)	62 ± 3.0 (23)	---
F0 Females				
0	118 ± 7.4 (23)	83 ± 7.2 (24)	75 ± 4.4 (23)	---
550	117 ± 6.5 (25)	87 ± 8.7 (20)	77 ± 6.8 (22)	---
1700	118 ± 6.6 (24)	87 ± 5.8 (22)	75 ± 7.0 (23)	---
5000	115 ± 6.6 (21)	86 ± 7.4 (23)	77 ± 8.6 (24)	---
7500	115 ± 6.8 (19)	84 ± 6.0 (22)	76 ± 7.1 (21)	---

Dietary Concentration (ppm)	Mean Food Consumption (g/kg/day ± SD) at Study Week: 31	34	37	40
F1 Males				
0	113 ± 14.1 (23)	82 ± 5.2 (24)	68 ± 3.4 (25)	59 ± 2.7 (25)
550	115 ± 16.0 (25)	82 ± 7.0 (24)	69 ± 4.4 (25)	59 ± 3.5 (24)
1700	109 ± 20.1 (24)	82 ± 4.6 (24)	68 ± 4.6 (25)	60 ± 2.7 (25)
5000	116 ± 22.4 (23)	85 ± 7.9 (23)	68 ± 4.8 (23)	59 ± 3.6 (24)
7500	129 ± 18.1* (23)	89 ± 10.5* (23)	72 ± 6.2* (24)	61 ± 2.7 (2[illegible])
F1 Females				
0	122 ± 16.2 (24)	97 ± 7.4 (23)	87 ± 7.2 (24)	78 ± 6.4 (24)
550	125 ± 21.6 (25)	98 ± 8.7 (25)	86 ± 9.2 (25)	76 ± 6.1 (25)
1700	116 ± 28.3 (25)	98 ± 7.6 (22)	83 ± 3.8 (24)	76 ± 4.5 (25)
5000	126 ± 26.8 (25)	96 ± 7.6 (19)	86 ± 10.0 (22)	78 ± 5.5 (2[illegible])
7500	153 ± 35.8* (21)	108 ± 17.1 (15)	93 ± 12.2 (21)	78 ± 6.5 (13)

[a]Data were extracted from study No. 87-3243, Tables 10 and 11.

[b]Number of animals used in parentheses.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 5. Summary of Maternal Food Consumption During Gestation in Rats Fed AC 6,601 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Food Consumption (g/kg/day ± S.D.) on Gestational Day: 0 - 7	7 - 14	14 - 20
F_0 generation - F_{1A} litter			
0	90 ± 9 (20)[b]	86 ± 13 (22)	82 ± 17 (22)
550	88 ± 10 (22)	88 ± 9 (20)	82 ± 9 (22)
1700	85 ± 11 (20)	85 ± 8 (22)	83 ± 10 (22)
5000	82 ± 9 (21)	88 ± 10 (23)	82 ± 6 (24)
7500	81 ± 13* (19)	85 ± 9 (22)	85 ± 10 (23)
F_0 generation - F_{1B} litter			
0	77 ± 8 (18)	75 ± 14 (19)	79 ± 10 (19)
550	81 ± 10 (19)	79 ± 11 (18)	81 ± 8 (19)
1700	79 ± 16 (20)	80 ± 6 (16)	81 ± 14 (17)
5000	80 ± 11 (18)	78 ± 9 (18)	79 ± 5 (18)
7500	84 ± 10 (17)	77 ± 9 (18)	79 ± 10 (20)
F_1 generation - F_{2A} litter			
0	91 ± 15 (19)	88 ± 7 (19)	84 ± 6 (19)
550	87 ± 9 (22)	83 ± 11 (22)	79 ± 13 (21)
1700	84 ± 6 (20)	83 ± 5 (17)	78 ± 10 (19)
5000	88 ± 14 (23)	83 ± 20 (20)	80 ± 10 (22)
7500	97 ± 14 (23)	88 ± 6 (12)	89 ± 14 (20)
F_1 generation - F_{2B} litter			
0	76 ± 8 (16)	76 ± 5 (16)	73 ± 6 (16)
550	76 ± 6 (20)	69 ± 4* (20)	70 ± 8 (20)
1700	75 ± 8 (17)	73 ± 8 (17)	71 ± 6 (17)
5000	79 ± 5 (13)	77 ± 7 (14)	75 ± 6 (14)
7500	77 ± 9 (15)	76 ± 7 (18)	80 ± 11 (19)

[a]Data were extracted from study No. 87-3243, Tables 28, 29.

[b]Number of animals used in parentheses.

*Significantly different from controls ($p \leq 0.05$).

In the F_0 generation, daily food consumption for males was significantly ($p \leq 0.01$ or 0.05) lower than controls during the premating period at 550 ppm during weeks 4 and 7, at 1700 ppm during weeks 4 and 6-8, and at 5000 ppm during week 6, and during the postmating period at 550 ppm during weeks 13 and 17-18, at 1700 ppm during weeks 13-15 and 17-18, and at 5000 ppm during weeks 13-14 and 17. For females, food consumption was significantly ($p \leq 0.01$ or 0.05) lower than controls at 5000 ppm during premating week 5 and at 7500 ppm on GD 0-7 during the first gestational period. Mean test material intake during the premating period was 0, 42.71, 131.79, 393.54, and 594.98 mg/kg/day for males and 0, 49.91, 151.89, 438.09, and 655.05 mg/kg/day for females in the 0-, 550-, 1700-, 5000-, and 7500-ppm groups, respectively.

In the F_1 generation, daily food consumption for males was significantly ($p \leq 0.01$ or 0.05) increased above controls during the premating period at 7500 ppm during weeks 33 and 34-39. For females, food consumption was significantly ($p \leq 0.01$ or 0.05) lower than controls at 5000 ppm during premating week 35, at 550 ppm on GD 7-14 (second litter), at 1700 ppm during week 51, at 5000 ppm during week 50, and at 7500 ppm during week 51 of the final rest period and significantly ($p \leq 0.01$ or 0.05) increased above controls at 7500 ppm during the premating period on week 31. Mean test material intake during the premating period was 0, 42.61, 129.97, 394.49, and 628.04 mg/kg/day for males and 0, 51.23, 154.25, 464.71, and 751.84 mg/kg/day for females in the 0-, 550-, 1700-, 5000-, and 7500-ppm groups, respectively.

Gross and Microscopic Pathology: No compound-related gross or microscopic findings were reported.

3. Reproductive Toxicity: The effects of dietary administration of the test material on reproductive parameters are summarized in Tables 6-9.

In the F_1 generation, the lactation index was significantly ($p \leq 0.05$) decreased during the first mating at 7500 ppm. Pup body weight was significantly ($p \leq 0.01$ or 0.05) decreased below controls in the first litters at 550, 5000, and 7500 ppm on lactational day 21 and in the second litters at 7500 ppm on lactational days 0 and 21.

In the F_2 generation, fertility indices for females (Table 9) and males (76, 86, 74, 73, and 83% in the control, 550-, 1700-, 5000-, and 7500-ppm groups, respectively) for F_{2B} litters were slightly reduced in all

TABLE 6. Summary of Effects of Dietary Administration of AC 6,601 on F_{1A} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Concentration (ppm) 0	550	1700	5000	7500
No. matings	25	24	25	25	24
No. pregnancies	24	23	23	24	24
Fertility index-female (%)	96	96	92	96	100
Gestation index (%)	100	100	100	100	100
Gestation length (days)	22.1	22.0	21.9	22.0	22.0
Total No. live pups					
Day 0	313	295	313	310	300
Day 4, precull	308	290	312	295	293
Day 21	189	180	183	183	179
Mean No. live pups/litter					
Day 0	13.0	12.8	13.6	12.9	12.5
Day 4, precull	12.8	12.6	13.6	12.3	12.2
Day 21	7.9	7.8	8.0	7.6	7.5
Live birth index (%)[b]	100	98	99	100	99
Viability index (%)[c]	98	98	99	95	98
Lactation index (%)[d]	99	100	99	97	94*
Mean pup body weight/litter (g)					
Day 0	6.2	5.9	5.9	5.8	5.8
Day 7	15.2	14.6	15.3	14.5	14.7
Day 21	46.3	42.5*	44.7	39.8**	39.4**
Sex ratio (m/f, day 0)	0.9	1.0	1.1	1.1	0.9

[a]Data were extracted from study No. 87-3243, Tables 35-39 and individual animal data.

[b]Calculated by the reviewers as: $\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100.$

[c]Viability index was calculated as: $\frac{\text{No. of pups alive on Day 4 preculling}}{\text{No. of pups born alive}} \times 100.$

[d]Lactation index was calculated as: $\frac{\text{No. of pups alive on Day 21}}{\text{No. of pups Day 4 postculling}} \times 100.$

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 7. Summary of Effects of Dietary Administration of AC 6,601 on F_{1B} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Concentration (ppm) 0	550	1700	5000	7500
No. matings	25	25	25	25	25
No. pregnancies	24	22	23	22	22
Fertility index-female (%)	96	88	92	88	88
Gestation index (%)	100	100	100	100	100
Gestation length (days)	22.1	21.9	21.9	22.0	22.3
Total No. live pups					
Day 0	297	303	283	268	293
Day 4, precull	286	277	292	249	259
Day 21	178	167	180	158	162
Mean No. live pups/litter					
Day 0	12.4	13.8	12.9	12.2	13.3
Day 4, precull	11.9	13.2	12.7	11.9	12.3
Day 21	7.4	8.0	7.8	7.5	7.4
Live birth index (%)[b]	93	99	97	96	96
Viability index (%)[c]	96	96	98	93	93
Lactation index (%)[d]	97	95	99	99	96
Mean pup body weight/litter (g)					
Day 0	6.0	5.8	6.1	5.6	5.5*
Day 7	13.8	13.7	14.7	14.3	13.1
Day 21	44.1	44.8	45.7	42.1	39.4**
Sex ratio (m/f, day 0)	0.9	1.2	1.1	0.9	1.1

[a]Data were extracted from study No. 87-3243, Tables 35-39 and individual animal data.

[b]Calculated by the reviewers as: $\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100.$

[c]Viability index was calculated as: $\frac{\text{No. of pups alive on Day 4 preculling}}{\text{No. of pups born alive}} \times 100.$

[d]Lactation index was calculated as: $\frac{\text{No. of pups alive on Day 21}}{\text{No. of pups Day 4 postculling}} \times 100.$

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 8. Summary of Effects of Dietary Administration of AC 6,601 on F_{2A} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Concentration (ppm) 0	550	1700	5000	7500
No. matings	24	25	25	24	25
No. pregnancies	24	25	24	24	25
Fertility index-female (%)	100	100	96	100	100
Gestation index (%)	100	100	96	100	100
Gestation length (days)	22.1	22.0	22.5	22.1	22.2
Total No. live pups					
Day 0	327	308	313	258	299
Day 4, precull	298	298	299	247	291
Day 21	180	194	179	162	191
Mean No. live pups/litter					
Day 0	13.6	12.3	13.0	11.2	12.0
Day 4, precull	13.0	11.9	13.0	11.2	11.6
Day 21	7.8	7.8	7.8	7.4	7.6
Live birth index (%)[b]	98	99	94	99	99
Viability index (%)[c]	91	97**	96	90	97**
Lactation index (%)[d]	99	99	98	98	98
Mean pup body weight/litter (g)					
Day 0	5.6	6.0	6.1*	6.0	5.9
Day 7	14.2	15.0	15.0	14.6	13.9
Day 21	45.0	46.2	47.7	43.3	40.5**
Sex ratio (m/f, day 0)	0.9	1.1	0.8	0.9	1.2

[a]Data were extracted from study No. 87-3243, Tables 37-40 and individual animal data.

[b]Calculated by the reviewers as: $\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100$.

[c]Viability index was calculated as: $\frac{\text{No. of pups alive on Day 4 preculling}}{\text{No. of pups born alive}} \times 100$.

[d]Lactation index was calculated as: $\frac{\text{No. of pups alive on Day 21}}{\text{No. of pups Day 4 postculling}} \times 100$.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 9. Summary of Effects of Dietary Administration of AC 6,601 on F_{2B} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Concentration (ppm)				
	0	550	1700	5000	7500
No. matings	22	25	24	23	24
No. pregnancies	17	20	18	1[illegible]	20
Fertility index-female (%)	77	80	75	70	83
Gestation index (%)	100	100	100	94	100
Gestation length (days)	22.1	21.9	22.3	22.4	22.1
Total No. live pups					
Day 0	191	268	228	158	248
Day 4, precull	181	266	213	138	240
Day 21	115	159	123	95	158
Mean No. live pups/litter					
Day 0	11.2	13.4	13.4	9.9	12.4
Day 4, precull	11.3	13.3	13.3	[illegible].9	12.0
Day 21	7.2	8.0	7.7	6.8	7.9
Live birth index (%)[b]	97	96	96	85	99
Viability index (%)[c]	95	99*	93	87*	97
Lactation index (%)[d]	100	100	98	100	100
Mean pup body weight/litter (g)					
Day 0	5.9	5.9	6.0	5.5*	5.8
Day 7	15.1	14.8	15.3	12.8*	15.0
Day 21	51.0	49.3	50.8	40.9**	44.0**
Sex ratio (m/f, day 0)	1.0	0.8	1.0	0.9	1.1

[a]Data were extracted from study No. 87-3243, Tables 37-40 and individual animal data.

[b]Calculated by the reviewers as: $\frac{\text{No. of live pups born}}{\text{No. live and dead pups born}} \times 100$.

[c]Viability index was calculated as: $\frac{\text{No. of pups alive on Day 4 preculling}}{\text{No. of pups born alive}} \times 100$.

[d]Lactation index was calculated as: $\frac{\text{No. of pups alive on Day 21}}{\text{No. of pups Day 4 postculling}} \times 100$.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

groups as compared to the other fertility indices. Gestation indices were slightly decreased during the first mating at 1700 ppm and during the second mating at 5000 ppm. Viability indices were significantly ($p \leq 0.01$) increased at 550 and 7500 ppm during the first mating and significantly ($p \leq 0.05$) increased at 550 ppm and decreased at 5000 ppm during the second mating. Pup body weight was significantly ($p \leq 0.01$ or 0.05) decreased in the first litter at 7500 ppm on lactational day 21 and in the second litter at 5000 and 7500 ppm on lactational days 7-21 and 21, respectively. Pup body weight was significantly ($p \leq 0.05$) increased at 1700 ppm (F_{2a}) on lactation day 0.

D. <u>REVIEWERS' DISCUSSION/CONCLUSIONS</u>:

1. <u>Test Material Analysis</u>: Stability (7 days frozen or at room temperature) of the test compound in the diet as confirmed. The initial homogeneity analysis of the high-dose diet was unacceptable; aliquots ranged from +17 to -10% of the mean concentration. However, subsequent analyses were within acceptable range, and therefore, the initial analysis was not considered by the reviewers impact negatively on the study. Concentrations of the test compound in the diet were within the acceptable range (±20%) of nominal concentrations.

2. <u>Parental Toxicity</u>: Slight increases (2%) in mortality were observed in parental animals during both generations, but a dose-related pattern was not evident. The study author gave no explanation for the deaths, but an intercurrent infection may have been possible, particularly since wide variations in temperature were observed during the study, and necropsy findings suggested pulmonary problems in found dead animals. The reviewers were unable to determine whether this impacted negatively on the study because individual clinical observations were not presented, and microscopic examinations were not performed on found dead animals.

   Significant compound-related reductions in body weight were observed at 7500 ppm in the F_0 females during the first gestational and lactational periods as well as in the F_1 males and females during the premating period. No consistent pattern, different from controls, was observed in food consumption among treated animals at any dose level. Therefore, the observed increases/ decreases in food consumption were not considered to be compound related.

   Based on the reduced body weights, the parental toxicity NOEL and LOEL were 5000 and 7500 ppm, respectively.

3. Reproductive Toxicity: Fertility, length of gestation, and pup viability were not affected by the test compound. The fertility indices for F_{1B} males and females were below those observed in the other matings. They were, however, not considered to be compound related since the reductions occurred in all groups, including the control group. Because there were no dose-related or consistent decreases within/across generations, the slight decreases observed in pup viability (reflected in gestation, live birth, viability, and lactation indices and caused by complete litter mortality in a single litter) in both generations at various dose levels were not considered to be compound related. Significant compound-related pup body weight reductions, however, were observed at 5000 and 7500 ppm during the lactational periods (particularly on day 21) in both generations. The significant reduction in body weight observed at 550 ppm in the F_{1A} pups on lactational day 21 was not considered to be compound related, since no such body weight reductions were observed in the other litters at this dose level.

Based on the reductions in pup body weight, the reproductive toxicity NOEL and LOEL were 1700 and 5000 ppm, respectively.

4. Study Deficiencies:

a. Individual clinical observation data were not presented.

b. Histopathology examination of found dead animals with gross lesions was not performed, and therefore, unexplained deaths could not be evaluated.

c. The desired temperature in the animal quarters (67-76°F) was greatly exceeded on 68/906 occasions (7.5%), and the actual temperature range was 57-83°F.

d. No historical control data were reported. The variability observed in pup viability may have been confirmed had this data been included.

E. CLASSIFICATION: CORE Minimum Data.

Parental Toxicity NOEL = 5000 ppm.

Parental Toxicity LOEL = 7500 ppm.

Reproductive Toxicity NOEL = 1700 ppm.

Reproductive Toxicity LOEL = 5000 ppm.

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F. <u>RISK ASSESSMENT</u>: Not applicable.