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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

AUG 05 1998

SUBJECT: Pentachlorophenol: review of a two-generation reproduction study in rats.

EPA Identification Numbers:

P.C. Code: 063001
DP Barcode: D242695
Submissions: S536665
MRID: 44464101
ID# 005382-00016

TO: Adam Heyward / Nader Elkassabany
PM Team # 34
Regulatory Management Branch II
Antimicrobials Division (7510W)

FROM: Timothy F. McMahon, Ph.D. 7/28/98
Senior Toxicologist, RASSB
Antimicrobials Division (7510W)

THRU: Laura Morris Laura Morris 8/5/98
Team Leader, Team Two
RASSB/AD (7510W)

And

Norm Cook, Chief Laura Morris for Norm Cook 8/5/98
RASSB/AD (7510W)

Registrant: Pentachlorophenol Task Force

Action Requested: Review of a two-generation reproduction study in rats submitted for pentachlorophenol.

Background

The registrant (Pentachlorophenol Task Force) submitted a two-generation reproduction study on a technical grade of pentachlorophenol (lot EL-064, purity 88.9%). The results of review by the Risk Assessment and Science Support Branch, Antimicrobials Division, is summarized below:

CITATION: Hoberman, A.M. (1997): Oral (Gavage) Two-Generation (One Litter Per Generation) Reproduction Study of Pentachlorophenol in Rats. Study performed by Argus Research Laboratories, Horsham, PA for the Pentachlorophenol Task Force. Submitted under MRID # 44464101. Unpublished.

EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 44464101), Pentachlorophenol (lot no. EL-064, purity 88.9%) in corn oil was administered to 30 male and female Sprague-Dawley rats/sex/dose by gavage at dose levels of 0, 10, 30, and 60 mg/kg/day. P1 male and female rats were given the test material once daily at least 70 days prior to cohabitation and continuing through the day before sacrifice. F1 generation rats may have been exposed *in utero* during gestation and via maternal milk during the postpartum period. The day after weaning, F1 generation rats were administered the test material by gavage at the same dose levels as P1 rats and were continued until one day prior to sacrifice. F2 pups were exposed possibly indirectly during maternal gestation or via maternal milk. In addition to standard parameters, estrous cycling and sperm morphology and function were measured. At 60 mg/kg/day pentachlorophenol, body weight of P1 and F1 parental rats was significantly decreased during the pre-mating interval (11-13% in P1 males, 7-8% in P1 females; 30% in F1 males, 16% in F1 females). Weight gain during pre-mating at 60 mg/kg/day pentachlorophenol was decreased 18% in P1 males, 16% in P1 females, 30% in F1 males, and 16% in F1 females. Gestational and lactational body weights of P1 and F1 female rats were also significantly decreased (19-22% in P1 and F1 females). Fertility index was decreased to 89% in high dose P1 male and female rats, and 81% in F1 male and female rats (vs 100% and 93% in controls, respectively). Number of litters were also decreased in both generations at 60 mg/kg/day pentachlorophenol (24 vs 27 in P1 controls; 17 vs 27 in F1 controls). Days to vaginal patency were significantly increased in P1 female pups at 60 mg/kg/day 936 vs 32 days in control), as was days to preputial separation in P1 male pups (49 vs 44 days in control). Estrous cycling was not significantly affected in either P1 or F1 females, but in F1

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males, the number of sperm observed with a broken flagellum was significantly increased at 60 mg/kg/day, (although the method of calculation was not clear) and the average testicular spermatid count was significantly decreased at 60 mg/kg/day. Testis weight in F1 generation males was also decreased. Group mean pup weight at 60 mg/kg/day pentachlorophenol was decreased up to 41% in P1 litters and 39% in P2 litters by day 21 post-partum. Group mean weight was also significantly decreased at 30 mg/kg/day I both generations of litters.
Decreased brain weight (4-8%) and increased liver weight (20-33%) were observed in both P1 and F1 male and female rats at 60 mg/kg/day. Macroscopic pathology (enlarged liver) and microscopic pathology (centrilobular hypertrophy, subacute inflammation, single cell necrosis, pigment deposition) were observed in increased incidence at 60 mg/kg/day. The single cell necrosis and pigment deposition were considered related to treatment. Mean litter size, number of live pups, and viability index were significantly reduced in the P1 and F1 pups. Decreased weight of the liver, brain, spleen, anf thymus were observed in F2 pups at 60 mg/kg/day.
At 30 mg/kg/day, body weight and weight gain decreases of approximately 11% were observed in F1 female parental rats. Significant decreases in average testicular spermatid count and testis weight were observed in F1 male parental rats.

Based on the data in this study, the Systemic NOEL = 10 mg/kg/day for male and female parental rats. The Systemic LOEL = 30 mg/kg/day for male and female rats, based on decreased body weight and weight gain in F1 generation parental rats, and adverse testicular effects in F1 male rats (decreased testis weight, decreased spermatid count).

The reproductive NOEL = 10 mg/kg/day in this study. The reproductive LOEL = 30 mg/kg/day, based on decreased group mean litter weight.

This reproductive study in the rat is classified **unacceptable** but can be upgraded pending submission of homogeneity data for the dosing solutions used in this study.Data submitted on homogeneity were from a different study.

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EPA Reviewer: Timothy F. McMahon, Ph.D. _____, Date 7/28/98
Senior Scientist, RASSB/AD (7510W)
EPA Secondary Reviewer: Roger Gardner, Ph.D. Roger Gardner Date 7/28/98
Toxicologist, BPPD

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - rat- OPPTS 870.3800 (§83-4)

DP BARCODE: D242695 SUBMISSION CODE: S536665
P.C. CODE: 063001

TEST MATERIAL (PURITY): Pentachlorophenol, CAS 87-86-5, brown chips. Purity of the test article was not reported.

SYNONYMS:

CITATION: Hoberman, A.M. (1997): Oral (Gavage) Two-Generation (One Litter Per Generation) Reproduction Study of Pentachlorophenol in Rats. Study performed by Argus Research Laboratories, Horsham, PA for the Pentachlorophenol Task Force. Submitted under MRID # 44464101. Unpublished.

SPONSOR: Pentachlorophenol Task Force.

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female rats, and 81% in F1 male and female rats (vs 100% and 93% in controls, respectively). Number of litters were also decreased in both generations at 60 mg/kg/day pentachlorophenol (24 vs 27 in P1 controls; 17 vs 27 in F1 controls). Days to vaginal patency were significantly increased in P1 female pups at 60 mg/kg/day 936 vs 32 days in control), as was days to preputial separation in P1 male pups (49 vs 44 days in control). Estrous cycling was not significantly affected in either P1 or F1 females, but in F1 males, the number of sperm observed with a broken flagellum was significantly increased at 60 mg/kg/day, (although the method of calculation was not clear) and the average testicular spermatid count was significantly decreased at 60 mg/kg/day. Testis weight in F1 generation males was also decreased. Group mean pup weight at 60 mg/kg/day pentachlorophenol was decreased up to 41% in P1 litters and 39% in P2 litters by day 21 post-partum. Group mean weight was also significantly decreased at 30 mg/kg/day I both generations of litters.
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At 30 mg/kg/day, body weight and weight gain decreases of approximately 11% were observed in F1 female parental rats. Significant decreases in average testicular spermatid count and testis weight were observed in F1 male parental rats.

Based on the data in this study, the Systemic NOEL = 10 mg/kg/day for male and female parental rats. The Systemic LOEL = 30 mg/kg/day for male and female rats, based on decreased body weight and weight gain in F1 generation parental rats, and adverse testicular effects in F1 male rats (decreased testis weight, decreased spermatid count).

The reproductive NOEL = 10 mg/kg/day in this study. The reproductive LOEL = 30 mg/kg/day, based on decreased group mean litter weight.

This reproductive study in the rat is classified **unacceptable** but can be upgraded pending submission of homogeneity data for the dosing solutions used in this study.

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COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Pentachlorophenol
   Description: brown chips
   Lot/Batch #: EL-064
   Purity: 88.9%
   CAS #: 87-86-5

2. Vehicle:
   Description: Mazola corn oil, a viscous liquid
   Lot/Batch #'s: 315A6, 613B6, 624A6, A10B6, 827A6, 924B6, 108A7
   Purity: N/A

3. Test animals: Species: rat
   Strain: Crl:CD®BR VAF/Plus® (Sprague-Dawley)
   Age at start of dosing: (P1): approximately 4 wks
   (F_1): not stated
   Weight at start of dosing:
   (P1) Males: 153-198g; Females: 132-159g
   (F_1) Males: 44-111g; Females: 30-100g
   Note: For the P1 generation rats, body weights were within an acceptable range (± 2 S.D.) At the start of the study. For the F1 parental rats, the range of body weights exceded 2 standard deviations for both sexes, based on mean body weight data given in the report (pages 363 and 493)

Source:

Housing: individually in stainless steel wire-bottomed cages, except during cohabitation and postpartum periods. During thee periods, each pair of male and female rats were housed in the male rat's cage. For delivery, each dam and litter were housed in a nesting box until weaning, when offspring were housed as described above for parents.

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Diet:Rats were given Certified Rodent Diet #5002 in individual feeders *ad libitum*.

Water: Local water filtered through a reverse osmosis membrane was supplied in individual water bottles *ad libitum*.

Environmental conditions:
Temperature: targeted for 70-78°F
Humidity: 40-70%
Air changes: minimum of 10 /hr
Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period: not stated

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Taken from the protocol, page 679 of the report: " Within each dosage group, consecutive order will be used to assign P1 generation rats to cohabitation, one male rat per female rat. A table of random units or a computer-generated random units will be used to assign F1 generation rats to cohabitation, one male rat to one female rat." " The cohabitation period (P1 and F1 generation rats) will consist of a maximum of 14 days. Female rats observed with spermatozoa in a vaginal smear and/or a copulatory plug will be considered to be at day 0 of presumed gestation.
After successful mating, female rats (beginning no later than gestation day 20) were housed individually in nesting boxes and were allowed to deliver. Each dam and litter were housed in a common nesting box during the postpartum period.

2. Study schedule: P1 generation rats were given the test material once daily at least 70 days prior to cohabitation and through one day prior to sacrifice, beginning at aproximately 6 weeks of age. Beginning on day 29 post-partum, F1 generation rats (offspring of P1) were administered the test article in the same manner as the P1 generation.

3. Animal assignment: Upon arrival, each P1 rat was assigned to individual housing using a computer-generated randomization procedure. After aclimation (stated as 7 days in the protocol), 120 rats/sex wre selected for study in the basis of physical appearance and body weight. P1 animals were randomly assigned to test groups as seen in Table 1.

TABLE 1 Animal Assignment

Test Group	Dose in Diet[a] *mg/kg/d*	Animals/group			
		P1 Males	P1 Females	F_1 Males	F_1 Females
0	0	30	30	30	30
10	10	30	30	30	30
30	30	30	30	30	30
60	60	30	30	30	30

4. Dose selection rationale: The doses for this study were, according to the report, selected "on the basis of previous studies conducted with the test substance." It is known that previos work with pentachlorophenol has involved these same dose levels, with effects usually noted at 30 and 60 mg/kg/day. Thus, the dose selection for this study does have scientific basis.

5. Dosage preparation and analysis

According to the protocol in the report (page 674), formulations of pentachlorophenol dosing solutions were prepared daily. Stability of the bulk test material was done by taking two 5 gram samples of the test material, one collected on the first day of dosing, and the second on the last day of treatment. During the course of the study, concentration of the test material in dosing solutions was analyzed by taking duplicate samples from the prepared formulations on the first and last day prepared. The results of this testing were found on pages 726-728 of the report. Homogeneity analyses were conducted on samples of pentachlorophenol dosing solutions obtained from another study. The results of these analyses are not considered applicable to this study. Stability analyses were conducted on samples of dosing solutions taken at each dose level from the present study. Over an 8-day period, the analytical results showed no significant degradation of pentachlorophenol in corn oil.

Concentration analyses were performed over the course of the study (samples taken 10/22/96, 2/25/97, and 6/19/97) at all dose levels (page 728 of the report). These data showed no significant deviations from the targeted dose levels at any of the doses tested over the course of the study.

The only deficiency noted here is the lack of acceptable homogeneity data for this study.

C. OBSERVATIONS

1. Parental animals: P1 animals were observed twice daily for viability and general appearance. Examinations for clinical signs, abortions, premature deliveries, and/or deaths were made approximately one hour before and one hour after administration of the test substance. Body weights were recorded weekly during acclimation, daily during dosing, and on the day of sacrifice for all rats. Food consumption for male rats was recorded weekly throughout the acclimation and dosing periods, and for female rats, weekly during acclimation and pre-mating periods. During gestation, foo consumption was recorded on gestation days 0, 6, 10, 15, 18, 20, and 25 and during lactation on days 1, 4, 7, 10, and 14.

P1 female rats were evaluated for estrous cycling for 22 days prior to cohabitation and continued until evidence of fertilization. Mating performance was evaluated daily during cohabitation and was confirmed by natural delivery of a litter or implantation sites observed at necropsy on gestation day 25. Vaginal cytology was performed on the day of scheduled sacrifice to determine the stage of the estrous cycle.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2 F_1/F_2 Litter Observations[a]

Observation	Time of observation (lactation day)					
	Day 1	Day 4[b]	Day 4[b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X

a Data extracted from Table C35 and E38 of the report.
b Before standardization (culling)
c After standardization (culling)

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

3. Postmortem observations:

1) Parental animals: All surviving P1 and F1 generation adults were sacrificed by carbon dioxide asphyxiation and examined for gross lesions. External and internal portions of hollow organs, external surfaces of the brain and spinal cord, nasal cavity and neck with associated tissues and organs, the thoracic, abdominal and pelvic cavities with associated organs and tissues, and the musculo/skeletal carcass were examined. Lungs were perfused with neutral buffered 10% formalin, while the urinary bladder and lungs from all postweaning rats were inflated with neutral buffered 10% formalin.

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

The following tissues (X) were prepared for microscopic examination. Organs marked (XX) were also weighed.

Organs Weighed	Organs examined Histologically
P1 Generation	**P1 Generation**
Uterus	Vagina
Ovaries	Uterus w/cervix
Brain	Ovaries w/oviducts
Liver	Liver
Kidneys	Right testis (left testis used for spermatid count)
Adrenals	Seminal vesicles
Spleen	Epididymides
Testis (left only)	
Epididymides	
Seminal Vesicles	
Prostate	
F1 Generation	**F1 Generation** Organs Examined

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Organs Weighed	Histologically
Uterus	Vagina
Ovaries	Uterus w/cervix
Brain	Ovaries[a] w/oviducts
Liver	Liver
Kidneys	Right testis (left testis used for spermatid count)
Adrenals	Seminal vesicles
Spleen	Epididymides
Testes	
Epididymides	
Seminal Vesicles	
Prostate	

F2 Generation Weanlings

Organs Weighed	Organs Examined Histologically
Brain	Brain
Liver	Liver
Spleen	Spleen
Thymus	Thymus
	Gross Lesions

[a]Five ovarian sections were taken at least 100μm apart from the inner third of each ovary. Examination included enumeration of the total number of primordial follicles from these 10 sections for comparison with control group values. Examination should also confirm the presence or absence of growing follicles and corpora lutea in comparison with control group ovaries.

2) Offspring: All F1 and F2 generation pups culled on lactation day 4 were sacrificed and examined for gross lesions. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the brain for hydrocephaly. F1 pups not selected for continued evaluation and F2 pups were sacrificed on lactation days 28 and 21, respectively. At least three pups per litter, when possible, were randomly selected, necropsied and examined for gross lesions. Gross lesions were retained in 10% neutral bufered formalin. All other pups were sacrificed without further evaluation.

D. DATA ANALYSIS

1. Statistical analyses: Paternal, maternal, and pup incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Body weight, food consumption, and litter averages for pup body weights, percent male pups and percent pup mortality were analyzed using Bartlett's Test of homogeneity of variances and analysis of variance when apropriate. If the analysis of variance was significant, Dunnett's Test was used to identify the statistical significance of the individual groups. If Bartlett's Test was significant, the Kruskal-Wallis Test was used when 75% or fewer ties were present; when more than 75% ties were present, Fisher's Exact Test was used. In cases where the Kruskal-Wallis Test was statistically significant, Dunn's Method of Multiple Comparisons was used to identify te statistical significance of the individual groups.

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Fertility index
Gestation index
No. and sex of offspring per litter
No. of implantation sites
Estrous cycling (P1 generation)
Sperm motility, morphology, and count
Spermatid Count

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

Litter size and viability on days 4, 7, 14, 21
Viability index
Lactation index
Percent survival and sex ratio

3. Historical control data: none provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs:

The following clinical signs were observed: Thes
results are summarized from the report in Table 3

TABLE 3 Mortality and Clinical Signs[a]

Observation	Dose Group (mg/kg/day			
	0	10	30	
[P1] Generation - Males				
swollen snout	0/0	8/1	0/0	
excess salivation	0/0	0/0	3/3	
[P1] Generation - Females				
excess salivation	0/0	0/0	2/1	
urine stained abdominal fur	0/0	0/0	0/0	
[F1] Generation Males				
found dead	0	0	2	
moribund sacrifice	0	0	2	
[F1] Generation Females				
found dead	0	0	1	
urine stained abdominal fur	11	6/2	0/0	
ungroomed coat	0/0	0/0	1/1	
soft or liquid feces	0/0	1/1	0/0	
corneal opacity	0/0	0/0	0/0	4
constricted pupil	0/0	0/0	0/0	4

a Data extracted from Tables B1 and C1, pages 80 and 184 of the report and from Tables D1 and E1, pages 361 and 489 of the report.(/) = Number of recorded incidents/no of rats observed with sign.

The report concluded that the clincal observations in this study for the P1 generation rats were unrelated to treatment. In the F1 generation, the report acknowledged that in females, a significantly increased incidence of urine-stained abdominal fur was observed at 60 mg/kg/day. The reviewer notes corneal opacity and constricted pupil in one female F1 rat at the 60 mg/kg/day dose.

The deaths in this study were explained as follows:

In the F1 generation, two deaths were observed in male rats at the 30 mg/kg/day dose level. One rat was sacrificed moribund on postweaning day 91. On the day of sacrifice, this rat was observed with red-stained abdominal fur, chromodacryorrhea, chromorhinorrhea, limited use of hindlimbs and cold to touch. Necropsy showed testes located in the abdominal cavity and red fluid in the urinary bladder. Histopathologic examination showed moderate hemorrhagic cystitis in the urinary bladder. The second rat was sacrificed moribund on postweaning day 49. This rat was observed with chromodacryorrhea, urine-stained abdominal fur, and red substance in the urine. Necropsy showed thickened rad walls of the urinary bladder and a dark green fluid within the blader. Histopathologic examination showed moderate hemorrhagic cystitis. A third rat was found dead at the 60 mg/kg/day dose level on postweaning day 2 immediately after dosing. Death was attributed to either failure to thrive or a dosing accident. Necropsy revealed no gross lesions.

In female F1 generation rats, one rat at 30 mg/kg/day and 3 rats at 60 mg/kg/day died prior to sacrifice. The 30 mg/kg/day rat was found dead on postweaning day 23, 61 minutes after dosing. No adverse signs were noted before death in this rat. Death was believed to be due to a dosing accident.
At 60 mg/kg/day, the three deaths were believed to be the result of intubation accidents. Only one rat was observed with abnormal gross necropsy (fluid-filled lungs, congested lungs).

2. Body weight and food consumption:

In P1 male parental rats, group mean body weight was decreased at the 30 and 60 mg/kg/day dose level vs control beginning on day 36 of treatment (data from the report page 81). This decrease was

minor, i.e. 4-6% from control. By premating day 70, group mean body weight in males at 30 mg/kg/day was decreased 6% from control, while group mean body weight in males at 60 mg/kg/day was decreased 11% vs control. On day 91 (first day following mating), group mean body weight in males at 30 mg/kg/day was decreased 6% vs control, and at 60 mg/kg/day, group mean body weight was decreased 13% from control. Between days 29-36 premating in male P1 rats, weight gain was decreased at the 30 and 60 mg/kg/day dose levels (7% and 21% respectively). For days 1-70 premating, weight gain in male P1 rats at 30 mg/kg/day was decreased 8%, and weight gain in males at 60 mg/kg/day was decreased 16% from control. For days 1-91, weight gain in male P1 rats at 30 mg/kg/day was decreased 8%, and weight gain in male P1 rats at 60 mg/kg/day was decreased 18%. Between day 1 and sacrifice, total weight gain in male P1 rats was decreased 8% at 30 mg/kg/day, and 18% at 60 mg/kg/day.
Food consumption in P1 male rats (on a g/day basis) was not significantly different in treated groups vs control except for days 57-64 premating, where food consumption at the 30 and 60 mg/kg/day dose levels was decreased 7% and 9%, respectively. On a per mg/kg/day basis, food consumption was unchanged in treated male rats vs control.

Reported body weight and selected food consumption results are summarized in Table 4.

TABLE 4 Body Weight and Food Consumption - Pre-matinga

Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
[P1] Generation Males - Pre-mating				
Mean body weight (g) Day 36	407.7± 32.7	397.1± 24.1	391.8± 23.2*	385.5± 31.1**
Day 70	509.8± 51.7	498.2± 38.8	480.9± 35.5*	456.5± 45**
Day 91	545.2± 57.5	534.6± 46.1	514.3± 37.0*	478.9± 49.8**
Mean weight gain (g) Days 1-70	333.8± 46.6	323.6± 34.7	307.1± 31.0*	282.3± 38.7**
Days 1-91	369.1± 52.6	360.0± 42.0	340.5± 33.0*	304.7± 42.9*
Day 1-sacrifice	375.8± 52.9	369.7± 42.9	347.4± 33.3*	311.3± 43.6**
Mean food consumption (g/animal/day) Days 1-70	23.5± 2.5	22.8± 2.2	22.1± 1.7	22.4± 2.0

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F1 Generation Males				
Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
Day 36	375.2± 34.3	358.0± 20.4	334.6± 31.0**	264.5± 41.6**
Day 71	513.4± 53.1	494.3± 37.5	459.6± 44.1**	358.6± 49.3**
Day 92	556.1± 58.7	536.6± 42.5	494.0± 53.2**	378.9± 50.4**
Mean weight gain (g) Days 1-71	421.9± 51.6	407.2± 35.2	382.5± 41.9**	302.1± 43.2**
Days 1-92	431.4± 54.2	415.5± 40.7	390.2± 45.4**	306.6± 43.4**
Days 1-sacrifice	499.2± 59.9	490.8± 52.9	452.8± 55.8**	354.6± 48.0**
Mean food consumption (g/animal/day) Days 1-71	23.0± 2.1	22.3± 1.4	21.5± 2.1*	18.2± 2.4**

a Data extracted from Tables B2, B3, D2, and D3, pages 81, 82, and 363-366 of the report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

In F1 male parental rats, group mean body weight was decreased at the 30 and 60 mg/kg/day dose level vs control from day 1 through to sacrifice. Generally, the weight decrease at 30 mg/kg/day was 11% from control, while the weight decrease at 60 mg/kg/day was 30% from control. Decreases in weight gain were similar as those for absolute group mean body weight in F1 males.
Food consumption in P1 male rats (on a g/day basis) was decreased by approximately 8% at the 30 mg/kg/day dose, and by approximately 20% at the 60 mg/kg/day dose, throughout the testing period. Thus, food consumption decreases could account for some, but not all, of the body weight changes observed in the F1 males. It is of interest that decreased body weight in F1 males was observed from day 1 of the study in contrast to P1 males, where changes were not observed until approximately day 36 of dosing.

Body weight and food consumption values for P1 female rats prior to cohabitation and F1 female rats postweaning to cohabitation are summarized below:

TABLE 5 Body Weight and Food Consumption - Pre-mating[a]

Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
[P1] Generation Females - Pre-mating				
Mean body weight (g) Day 36	234.4± 17.2	229.5± 20.0	225.6± 17.8	225.0± 19.2
Day 70	262.2± 21.1	259.1± 24.4	254.5± 23.2	241.8± 22.0**
Mean weight gain (g) Days 1-70	113.3± 18.6	111.4± 22.4	108.8± 19.9	95.3± 19.1**
Mean food consumption (g/animal/day) Days 1-70	16.5± 1.9	16.2± 1.6	15.7± 1.4	16.7± 2.7
F1 Generation Females Post-weaning (pre-cohabitation)				
Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
Day 1	84.1± 8.4	79.6± 7.9	70.8± 10.0	52.3± 9.3**
Day 36	211.8± 16.6	204.7± 16.7	200.7± 15.8*	173.3± 21.4**
Day 71	258.7± 23.0	252.1± 27.0	247.4± 19.4	217.7± 23.0**
Mean weight gain (g) Days 1-71	174.6± 21.5	172.6± 25.2	177.0± 18.4	164.7± 18.6
Days 1-precohabitation	177.0± 20.1	176.1± 26.2	178.5± 18.1	164.7± 21.4
Mean food consumption (g/animal/day) Days 1-71	15.4± 1.3	15.2± 1.6	15.3± 1.5	14.3± 1.7*

a Data extracted from Tables C2, C8, E2, and E8, pages 189, 198, 494, and 501 of the report. N = 30 for all dose groups.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

17
22

In P1 female rats prior to cohabitation, treatment with pentachlorophenol did not produce detrimental effects on body weight at 30 mg/kg/day. At 60 mg/kg/day, an effect was noted beginning (based on available data) at day 50 of dosing. Group mean body weight was decreased by 7-8% at this dose level from day 50 to day 70. Group mean weight gain for the 1-70 day preocohabitation period was decreased by 16% from control at the 60 mg/kg/day dose, with no effect observed at the 30 mg/kg/day dose.

Food consumption in P1 female rats was not affected by treatment with pentachlorophenol, in contrast to male rats. Thus, decreases in body weight and weight gain at the 60 mg/kg/day dose level can be supported as a treatment-related effect, as the basis for decreased body weight would not be based on decreased food consumption, but likely an effect on food efficiency. This is supported by the data (page 199 of the report) indicating an increase in relative food consumption at the high dose for female rats (g/kg/day). This observation, with the observation that body weight was unaffected or decreased, suggests that high dose rats were taking in greater amounts of food to maintain body weight in comparison to controls. The effect of pentachlorophenol as an uncoupler of oxidative phosphorylation could be a contributoing factor to this.

In F1 female rats post-weaning, group mean body weight at day 1 was decreased by 38% at the 60 mg/kg/day dose vs control. By day 70, group mean body weight at this dose was decreased by only 16%. However, weight gain for the entire dosing period in F1 female rats was not significantly affected. Food consumption for the dosing period in F1 female rats was decreased at the 60 mg/kg/day dose level by 8%.

In comparison to male rats pre-mating, the data suggest that female rats were not as sensitive to the effects of pentachlorophenol on body weight and food consumption as males. Although F1 females showed body weight deficits at post-weaning day 1, these deficits did not persist.

Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in the report as follows:

TABLE 6 Body Weight and Food Consumption - Gestationa

Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
[P1] Generation Females - Gestation				
Mean body weight (g) Day 1	266.8± 23.0	263.6± 24.8	258.9± 20.5	248.3± 25.9**
Day 10	296.2± 20.1	292.7± 24.1	288.6± 19.4	275.2± 29.1**
Day 20	378.7± 29.6	378.6± 24.2	361.8± 31.5	339.8± 38.8**
Mean weight gain (g) Days 0-20	116.3± 24.2	119.5± 13.4	106.4± 23.7	94.9± 29.1*
Mean food consumption (g/animal/day) Days 1-70	18.7± 2.3	19.1± 2.2	19.1± 1.6	19.8± 2.5
F1 Generation Females - Gestation				
Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
Day 1	259.2± 21.4	257.7± 23.9	244.8± 20.1*	211.9± 29.0**
Day 10	293.6± 24.2	288.0± 27.0	280.4± 24.2	240.5± 34.4**
Day 20	375.3± 29.5	361.9± 28.9	357.1± 31.0	295.4± 42.0**
Mean weight gain (g) Days 0-20	116.1± 20.6	104.2± 17.8	112.3± 26.4	83.5± 17.7
Days 1-precohabitation	177.0± 20.1	176.1± 26.2	178.5± 18.1	164.7± 21.4
Mean food consumption (g/animal/day) Days 1-71	15.4± 1.3	15.2± 1.6	15.3± 1.5	14.3± 1.7*

a Data extracted from Tables C2, C8, E2, and E8, pages 189, 198, 494, and 501 of the report. N = 30, except for 30 mg/kg/day dose, where N = 29.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

During gestation, body weight effects were observed in both the P1 and F1 female rats. In P1 females at 60 mg/kg/day, group mean body weight on day 20 of gestation was decreased 11% from control, and weight gain for the gestation period (days 0-20) decreased 19% from control. In F1 female rats, group mean body weight in gestation day 20 was decreased 22% from control, and weight gain for the 0-20 day gestation period decreased 29% from control. It is noted that the body weight effects in the F1 generation were more significant than those observed in the P1 generation, indicating possible greater toxicity to F1 than P1 females during gestation, possibly as a result of continued exposure of the F1 generation. Food consumption was not greatly affected in either generation during gestation, suggesting an effect of test article on food conversion.

TABLE 7 Body Weight and Food Consumption - Lactation

Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60
[P1] Generation Females - Lactation				
Mean body weight (g) Day 1	294.7± 18.1	291.8± 24.9	290.4± 24.5	276.4± 23.8**
Day 14	326.3± 18.0	321.8± 21.5	319.0± 20.8	295.0± 26.8**
Day 28	295.7± 16.3	290.0± 18.5	287.3± 16.5	277.1± 21.5**
Mean weight gain (g) Days 1-28	1.0± 11.3	-1.8± 13.9	-3.6± 17.1	0.7± 10.9
Mean food consumption (g/animal/day) Days 1-14	36.8± 4.9	37.3± 4.4	33.6± 7.3	29.0± 4.6**
F1 Generation Females - Lactation				
Observations/study week	Dose Group (mg/kg/day)			
	0	10	30	60

20

25

Day 1	295.8± 25.2	285.6± 26.4	281.2± 25.4	239.4± 31.6**
Day 14	319.6± 21.4	314.4± 23.2	310.0± 23.4	262.2± 34.8**
Day 21	321.0± 16.9	312.5± 25.5	304.3± 22.5*	264.2± 34.0**
Mean weight gain (g) Days 1-21	25.2± 15.9	26.9± 20.5	22.5± 16.2	22.3± 11.8
Mean food consumption (g/animal/day) Days 1-14	37.3± 7.3	37.1± 4.4	36.2± 5.2	25.7± 5.9**

a Data extracted from Tables C6, C7 C8, C12, E6, E7, E8, and E12, pages 194-197, 202, 498-501, and 505 of the report. N = 30, except N = 29 at 30 mg/kg/day and N = 26 at 60 mg/kg/day for F1 females, and n = 29 for P1 females at 30 mg/kg/day.

* Statistically different from control, $p<0.05$.

** Statistically different from control, $p<0.01$.

3. Test Substance Intake: Concentration analyses of dosing solutions used in this study were performed over the course of the study, and results were presented on page 728 of the report. Analyses were conducted on 10/22/96 (start of study), 2/25/97 (approximate time of start of F1 dosing), and on 6/19/97 (approximate time of sacrifice of F1 generation pups). Each dosage level was analyzed at these time points. For the dose levels of 0, 10, 30, and 60 mg/kg/day (corresponding to a target dose solution concentration of 0, 2, 6, and 12 mg/mL [5ml/kg dose volume]), the analyzed values did not deviate significantly from the target dose solution concentrations. Thus, it appears that dose solutions were mixed to the proper concentration.

4. Reproductive function:

a. Estrous cycle length and periodicity: For the P1 generation female rats, the mean number of estrous stages per 21 days was not significantly different in treated females vs. controls (4.9, 5.3, 4.3, and 5.3 for the 0, 10, 30, and 60 mg/kg/day dose groups, respectively). Results of vaginal smears showed no significant treatment-related effects on the number of rats in proestrus, estrus, and metestrus. The number of rats in diestrus appeared decreased at the 30 and 60 mg/kg/day dose levels (13 rats each dose, vs 19 in control and low dose rats).

For the F1 generation females, results of estrus cycling analyses showed no significant effects of treatment on estrus stages over 14 days, or in the number of rats in various stages of estrus. Significant increases in the number of female rats in estrus at the 10 and 60 mg/kg/day dose levels (7 and 5 rats, respectively, vs 1 in control) was observed, but a similar increase in this parameter was not observed at the 30 mg/kg/day dose level.

b. Sperm measures: In P1 generation males, there were no significant differences from control in the total number of motile sperm, total number of non-motile sperm, or in the total % motile sperm in any treatment group. Cauda epididymal sperm count was not significantly different among control and treated male P1 rats. Testicular spermatid count and concentration were also unaffected in treated male P1 rats at any dose level. Sperm morphology was also unaffected by treatment at any dose level.

In F1 generation males, total caudal epididymal motile sperm, total nonmotile sperm, and total percent motile sperm were not significantly affected by pentachlorophenol treatment. The number of sperm observed with a broken flagellum was significantly increased at 60 mg/kg/day (1.5 ± 1.6 vs 0.6 ± 1.4 in control), and the average testicular spermatid count was significantly decreased at the 30 and 60 mg/kg/day dose levels (157 ± 28.3 and 152.1 ± 32.2 respectively) vs control (175.8 ± 32.5). Testis weight in F1 generation males was decreased at the 30 and 60 mg/kg/day dose levels (1.5667 and 1.3315 grams vs 1.6634 grams in control).

c. Sexual maturation (F_1): Data summarizing effects of pentachlorophenol treatment on vaginal patency and fertility were presented in Tables E14 ad E15, pages 507-509 of the report. The average day of vaginal patency was significantly increased to 36.2 ± 3.0 days at 60 mg/kg/day pentachlorophenol, vs 32.0 ± 0.9 days in control ($p < 0.01$). In fact, days to vaginal patency were significantly increased at all dose levels from control (33.2 ± 1.1 and 33.9 ± 1.8 days at 10 and 30 mg/kg/day pentachlorophenol, respectively). However, according to the report, the values observed at these lower doses were within the historical control range of the performing laboratory, with a reported historical range of 30.1 - 33.7 in 462 control female rats from 16 studies conducted from March 1995 to September 1997.

Preputial separation in F1 male rats was significantly increased at 30 and 60 mg/kg/day pentachlorophenol (46.2±2.1 and 49.6±3.6 days, respectively, vs 44.5±2.0 days in control).

5. Reproductive performance: Results for the parental animals are summarized from the report in Table 5.

TABLE 5a Reproductive Performance[a]

Observation	Dose Group (ppm)			
	Control	LDT	MDT	HD
P1 Generation				
Mean precoital interval (days)	3.1±3.3	2.9±3.2	2.5±2.6	3.0±2.4
MALES				
Mated/total rats	28/30	29/30	28/29	29/30
Fertility index (%)	100	93	96	89
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated/total rats	28/30	29/30	28/29	29/30
Fertility Index (%)	100	93	96	89
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	1	0
Median gestation interval (days)	22.9±0.3	22.8±0.4	22.9±0.5	23.1±0.3

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Number of litters	27	27	27	24

a Data extracted from Tables B6, C14, and C19, pages 85, 204, and 211 of the report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

TABLE 5b Reproductive Performance[a]

Observation	Dose Group (mg/kg/day)			
	Control	10	30	60
F1 Generation				
Mean precoital interval (days)	3.3±2.9	4.0±3.3	2.9±1.8	5.0±5.0
MALES				
Mated/total rats	29/30	28/30	29/29	21/26**
Fertility index (%)	93	89	93	81**
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	1	1
FEMALES				
Number mated/total rats	29/30	28/30	29/29	21/26**
Fertility Index (%)	93	89	93	81**
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	1	3
Median gestation interval (days)	23.2±0.4	23.0±0.3	23.1±0.4	23.4±0.6
Number of litters	27	25	27	17

a Data extracted from Tables D7, E15, and E20, pages 372, 509, and 514 of the report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

5. Parental postmortem results

a) Organ weights: In P1 generation male rats, the following organ weight changes were observed:

i) reduced group mean body weight at 60 mg/kg/day vs control by 13%.

ii) reduced brain weight at 60 mg/kg/day (2.185g vs 2.254g in control, a decrease of 4%).

iii) increased absolute liver weight (33%) and kidney weight (10-11%).

In P1 generation female rats, the following was observed:

i) decreased group mean body weight at 60 mg/kg/day (7%)

ii) decreased brain weight (8%) at 60 mg/kg/day

iii) increased absolute liver weight (32%) at 60 mg/kg/day

iv) decreased ovary weight (31-36%) at 60 mg/kg/day

In F1 generation male rats, the following was observed:

i) decreased group mean body weight at 60 mg/kg/day (31%)

ii) decreased weight of the left epididymis (20%), left cauda epididymis (27%), left testis (19%), seminal vesicles (17%), right epididymis (16%), right testis (20%), prostate (31%), and brain (11%).

iii) increased absolute liver weight (14%) and kidney weight (11%).

iv) increased adrenal weight (11%), and spleen weight (17%)

In F1 generation female rats, the following was observed:

i) decreased group mean body weight (20%) at 60 mg/kg/day).

ii) decreased weight of the brain (9%), kidneys (17%), and left ovary (25%).

In both sexes of both generations, relative organ weights were also similarly affected as noted above for absolute organ weights. These changes in relative organ weights were due to decreased group mean body weight. It is noted that several significant changes in relative organ weights were noted at the 30 mg/kg/day dose level for both generations, thus supporting the 10 mg/kg/day dose as a no effect level for this effect.

b) Pathology

1) Macroscopic examination:

In P1 generation male rats, enlarged liver was noted in 8/30 rats at the 60 mg/kg/day dose of pentachlorophenol and in 2/30 rats at 30 mg/kg/day pentachlorophenol, vs 0/30 in control (Table B7 of the report). In P1 generation female rats (Table C15 of the report), there were by contrast no significant gross necropsy observations reported.

In F1 generation male rats, an increased incidence of rats (3/30) was recorded with small prostate (vs 0/30 in other treatment groups and control). In F1 generation females, there were no significant treatment related observations from gross necropsy.

2) Microscopic examination:

Histopathological evaluation was performed by Research Pathology Services, Inc., New Britain, PA for Argus Research Laboratories.

TABLE 6 Microscopic Histopathology[a]

Observation	Dose Group (mg/kg/day)			
	Control	10	30	60
P1 Generation				
MALES				
Liver - no. Examined	30	29	30	30
Centrilobular Hypertrophy-total	0	22	30	30
-Minimal	0	20	6	1
-Mild	0	2	13	7
-Moderate	0	0	11	22
Subacute, Centrilobular, Multifocal Inflammation-total	0	2	6	17
-Minimal	0	2	6	16
-Mild	0	0	0	1
Single Cell Necrosis-Total	0	1	4	21
-Minimal	0	1	4	18
-Mild	0	0	0	3
Centrilobular Pigment,minimal	0	0	4	11
FEMALES				
Liver - no. Examined	30	30	29	30
Centrilobular Hypertrophy-total	0	13	29	30
-Minimal	0	11	4	0
-Mild	0	2	10	3
-Moderate	0	0	15	25
Subacute, Centrilobular, Multifocal Inflammation-total	0	4	18	19
-Minimal	0	4	16	17
-Mild	0	0	2	0

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Single Cell Necrosis-Total	0	1	11	24
-Minimal	0	0	9	22
-Mild	0	1	2	2
Centrilobular Pigment-Total	0	3	13	26
-Minimal	0	2	10	15
-Mild	0	1	2	9
-Moderate	0	0	1	2
Bile Duct Proliferation	0	0	0	8
-Minimal	0	0	0	6
-Mild	0	0	0	2

a-data taken from pages 814-817 of the report.

As noted, for the P1 generation of both sexes, the liver showed significant non-neoplastic histopathologic alterations as a result of pentachlorophenol treatment. The report stated that the hepatocellular centrilobular hypertrophy and vacuolation were expected metabolic effects resulting from oxidative stress, common to several organochlorine compounds. The single cell necrosis and centrilobular pigment observed in increased incidence at 30 and 60 mg/kg/day pentachlorophenol were considered distinct alterations as a result of pentachlorophenol treatment. The report did not discuss nor mention the observation of increased bile duct proliferation in female rats at 60 mg/kg/day pentachlorophenol.

Histopathologic observations in F1 generation rats are summarized in the following table:

TABLE 7 Microscopic Histopathology[a]

Observation	Dose Group (mg/kg/day)			
	Control	10	30	60
F1 Generation				
MALES				
Liver - no. Examined	30	30	30	30
Centrilobular Hypertrophy-total	0	27	29	28
-Minimal	0	17	2	2

-Mild	0	7	12	9
-Moderate	0	3	14	17
-Marked	0	0	1	0
Subacute, Centrilobular, Multifocal Inflammation-total	0	4	9	12
-Minimal	0	4	9	9
-Mild	0	0	0	3
Single Cell Necrosis-Total	0	0	4	14
-Minimal	0	0	4	10
-Mild	0	0	0	4
Centrilobular Pigment-total	0	0	3	13
-Minimal	0	0	3	10
-Mild	0	0	0	3
Epididymis: mononuclear cell infiltration, focal/multifocal	10	19	28	28
-Minimal	10	17	22	21
-Mild	0	2	6	6
-Moderate	0	0	0	1
Necrotic Germ Cells	0	0	0	2
-Minimal	0	0	0	1
-Moderate	0	0	0	1
FEMALES				
Liver - no. Examined	30	30	30	30
Centrilobular Hypertrophy-total	0	4	27	27
-Minimal	0	4	17	1
-Mild	0	0	8	4
-Moderate	0	0	2	19
-Marked	0	0	0	3
Subacute, Centrilobular, Multifocal Inflammation-total	0	6	8	14
-Minimal	0	6	7	12

-Mild	0	0	1	2
Single Cell Necrosis-Total	0	0	4	11
-Minimal	0	0	4	10
-Mild	0	0	0	1
Centrilobular Pigment-Total	0	1	5	12
-Minimal	0	1	3	7
-Mild	0	0	1	4
-Moderate	0	0	1	1
Bile Duct Proliferation	0	0	0	5
-Minimal	0	0	0	3
-Mild	0	0	0	2

a-data taken from pages 814-817 of the report.

As with the P1 generation of rats, pentachlorophenol treatment produced significant liver pathologic alterations in the F1 generation rats of both sexes. These alterations included centrilobular hepatocellular hypertrophy and inflammation, single cell necrosis, and pigment deposition. The hepatocellular hypertrophy and inflammation was observed in increased incidence at all dose levels tested, while single cell necrosis and pigment deposition appeared in increased incidence at the 30 and 60 mg/kg/day dose levels. Not observed in the P1 generation but present in the F1 generation male rats at 30 and 60 mg/kg/day pentachlorophenol was focal or multifocal mononuclear inflammatory cell infiltration of the epididymides, and, at 60 mg/kg/day, necrotic germ cells. As with P1 generation rats, female F1 rats showed similar liver histopathology as well as increased incidence of bile duct cell proliferation at 60 mg/kg/day pentachlorophenol.

B. OFFSPRING

1. Viability and clinical signs: Table C21 of the report summarized clinical observations in F1 pups, while Table E22 sumarized similar observations. For F1 pups, the data showed an increased incidence of discolored muzzle at 30 mg/kg/day (in 4 litters over 8 days)but not 60 mg/kg/day, and no other clinical signs of significance. For F2 pups,

an increased incidence of purple snout was observed at 60 mg/kg/day (3 litters over 5 days) but no other significant clinical observations were noted.

Mean litter size and viability results from pups during lactation are summarized from the report in Table 8.

TABLE 8 Mean Litter Size and Viability[a]

Observation	Dose Group (mg/kg/day)			
	Control	10	30	60
	P_1 Generation			
Mean litter size				
Day 1	12.4±3.1	13.0±2.8	10.7±4.1	9.4±3.1**
Day 4[b]	12.3±3.1	12.9±2.8	10.7±4.0	9.1±3.0**
Day 4[c]	7.7±1.1	7.9±0.8	7.1±2.1	7.2±1.4
Day 14	7.7±1.1	7.8±0.8	7.3±1.8	7.2±1.4
Day 21	7.7±1.1	7.8±0.8	7.3±1.8	7.2±1.4
Number live pups				
Day 1	335	355	290	226
Day 4[b]	333	350	289	219
Day 4[c]	209	211	193	174
Day 14	208	211	191	173
Day 21	208	211	191	173
Number deaths				
Days 0-4	2	5	1	7
Days 4-21	1	0	0	1
Survival indices				
Viability index	98.8	98.0	99.3	94.4**
Lactation index	99.5	100.0	98.4	99.4

a Data extracted from Tables C20 and C35, pages 212-214 and 309-312 of the report.
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

TABLE 9 Mean Litter Size and Viability[a]

Observation	Dose Group (mg/kg/day)			
	Control	10	30	60
	F_1 Generation			
Mean litter size				
Day 1	12.4±3.5	11.8±2.9	11.7±3.2	6.9±2.9**
Day 4[b]	12.3±3.6	11.8±2.9	11.4±3.1	7.0±2.8**
Day 4[c]	7.6±1.4	7.8±0.7	7.6±1.2	6.3±2.0*
Day 14	7.5±1.4	7.8±0.7	7.4±1.6	6.4±1.6*
Day 21	7.5±1.4	7.8±0.7	7.4±1.6	6.3±1.5*
Number live pups				
Day 1	338	296	315	111
Day 4[b]	332	296	308	105
Day 4[c]	204	196	205	94
Day 14	203	196	201	83
Day 21	203	196	201	82
Number deaths				
Days 0-4	6	0	8	18
Days 4-21	1	0	6	4
Survival indices				
Viability index	98.2	100.0	97.5	85.4**
Lactation index	99.5	100.0	97.1	95.3**

a Data extracted from Tables E20 and E38, pages 514-516 and 623-626 of the report.
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

For the F1 generation pups, several indices were significantly affected at the 60 mg/kg/day pentachlorophenol dose level. Mean litter size, number of live pups, pup deaths days 0-4, viability index, and lactation index were all significantly decreased in relation to lower doses and control values. The same indices were affected in the P1 generation pups, but not to the same degree as in the F1 generation pups. These effects might possibly be related to the more severe effects of pentachlorophenol treatment

on reproductive indices in F1 male and female parental rats than in the P1 generation parental rats.

2. Body weight: Selected mean pup body weight data are presented in Table 9.

TABLE 9 Mean Litter Weight[a]

Day of lactation	Dose Group (mg/kg/day)			
	Control	10	30	60
	F_1 Generation			
Day 0	6.3±0.6	6.2±0.4	6.0±0.6	5.6±0.5**
Day 4[b]	8.6±1.0	8.5±0.8	7.7±1.0**	6.8±0.9**
Day 4[c]	8.6±1.0	8.5±0.8	7.7±1.0**	6.8±0.9**
Day 7	14.4±1.9	13.9±1.1	12.3±1.6**	9.2±1.5**
Day 14	30.0±4.9	29.6±2.4	26.3±3.4**	18.4±2.4**
Day 21	45.5±6.0	44.9±4.3	39.8±3.9**	28.1±3.9**

a Data extracted from Table E21, page 517 of the report.
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

3. Offspring postmortem results:

a) Organ weights: As summarized in Table C22, page 216 of the report, the necropsy of P1 litters (268, 284, 225, and 149 pups evaluated at the 0, 10, 30, and 60 mg/kg/day dose, respectively) showed no significant macroscopic or microscopic abnormalities. In the F1 generation pups, a white area was observed in the left cerebral cortex of one high dose pup, but not in any other treatment group.

Pup organ weights for the F2 male pups showed at 60 mg/kg/day reduced weight of the liver (1.19 grams vs 1.77 in control), spleen (0.09g vs 0.18g in control), brain (1.38g vs 1.52 g in control), and thymus (0.1g vs 0.2g in control). At 30 mg/kg/day, significant reductions in brain weight (1.48g) and thymus weight (0.16g) were observed in males. In female F2 pups, a similar pattern of decreased organ weights was observed as that for males. At 60 mg/kg/day, liver weight was reduced (1.14g vs 1.78g in control), as was spleen weight (0.08g vs 0.18g in control),

brain weight (1.33g vs 1.47g in control), and thymus weight (0.1g vs 0.2g in control). Brain weight at 30 mg/kg/day was reduced (1.43g) as was thymus weight (0.16g). The decreases in organ weight for F2 pups as mentioned were in all cases statistically significant.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS:

The investigators of this study concluded that a No Observable Adverse Effect Level of 10 mg/kg/day was applicable, with effects on reproduction observed at the 30 and 60 mg/kg/day dose levels. A reproductive NOEL was determined to be 10 mg/kg/day. The investigators did not specifically discuss a Systemic NOEL and LEL for this study. However, a NOEL of 10 mg/kg/day can be supported for systemic toxcity as well, base on observations of toxcity at 30 mg/kg/day in parental rats.

B. REVIEWER'S DISCUSSION:

In this study, pentachlorophenol (88.9%) in corn oil was administered to 30 male and female Sprague-Dawley rats/sex/dose by gavage at dose levels of 0, 10, 30, and 60 mg/kg/day. P1 male and female rats were given the test material once daily at least 70 days prior to cohabitation and continuing through the day before sacrifice. F1 generation rats may have been exposed *in utero* during gestation and via maternal milk during the postpartum period. The day after weaning, F1 generation rats were administered the test material by gavage at the same dose levels as P1 rats and were continued until one day prior to sacrifice. F2 pups were exposed possibly indirectly during maternal gestation or via maternal milk. In addition to standard parameters, estrous cycling and sperm morphology and function were measured.
Clinical signs were reported at the 60 mg/kg/day dose level in both P1 and F1 parental rats. In P1 parental rats, excessive salivation and swollen snout were observed in increased incidence in both male and females. In F1 parental rats, urine stained abdominal fur in 4 F1 female rats and corneal opacity and constricted pupil in 1 F1 female rat was observed at 60 mg/kg/day pentachlorophenol. Excessive salivation was also observed in a few male and female rats of the F1 generation.

Mortality was observed in this study in the F1 generation. Two deaths were observed in male rats at the 30 mg/kg/day dose level.

One rat was sacrificed moribund on postweaning day 91. On the day of sacrifice, this rat was observed with red-stained abdominal fur, chromodacryorrhea, chromorhinorrhea, limited use of hindlimbs and cold to touch. Necropsy showed testes located in the abdominal cavity and red fluid in the urinary bladder. Histopathologic examination showed moderate hemorrhagic cystitis in the urinary bladder. The second rat was sacrificed moribund on postweaning day 49. This rat was observed with chromodacryorrhea, urine-stained abdominal fur, and red substance in the urine. Necropsy showed thickened rad walls of the urinary bladder and a dark green fluid within the blader. Histopathologic examination showed moderate hemorrhagic cystitis. A third rat was found dead at the 60 mg/kg/day dose level on postweaning day 2 immediately after dosing. Death was attributed to either failure to thrive or a dosing accident. Necropsy revealed no gross lesions.

In female F1 generation rats, one rat at 30 mg/kg/day and 3 rats at 60 mg/kg/day died prior to sacrifice. The 30 mg/kg/day rat was found dead on postweaning day 23, 61 minutes after dosing. No adverse signs were noted before death in this rat. Death was believed to be due to a dosing accident.
At 60 mg/kg/day, the three deaths were believed to be the result of intubation accidents. Only one rat was observed with abnormal gross necropsy (fluid-filled lungs, congested lungs).

The study concluded that the deaths in this study were unrelated to the test article specifically, and were instead the result of dosing errors. There appears to be some dose-response relationship for mortality in female F1 rats but not in males. While the two deaths in male P1 rats at the 30 mg/kg/day dose level were preceded by clinical signs that are consistent with those observed in the study, a similar situation was not encountered at the 60 mg/kg/day dose. The deaths in this study may be related to an artefact of treatment rather than the treatment itself. Other studies with pentachlorophenol (most notably, the recent 2-year carcinogenicity study in rats conducted by the National Toxicology Program) have shown mortality at doses of 30 mg/kg/day pentachlorophenol and above.

Effects of pentachlorophenol treatment on body weight were observed at the 30 and 60 mg/kg/day dose levels in this study in both P1 and F1 generation rats. For days 1-70 premating, weight gain in male P1 rats at 30 mg/kg/day was decreased 8%, and weight gain in males at 60 mg/kg/day was decreased 16% from control. For days 1-91, weight gain in male P1 rats at 30 mg/kg/day was decreased 8%, and weight gain in male P1 rats at 60 mg/kg/day was decreased 18%. Between day 1 and sacrifice, total weight gain in

male P1 rats was decreased 8% at 30 mg/kg/day, and 18% at 60 mg/kg/day. In F1 male parental rats, group mean body weight was decreased at the 30 and 60 mg/kg/day dose level vs control from day 1 through to sacrifice. Generally, the weight decrease at 30 mg/kg/day was 11% from control, while the weight decrease at 60 mg/kg/day was 30% from control. Decreases in weight gain were similar as those for absolute group mean body weight in F1 males. Food consumption in P1 male rats (on a g/day basis) was decreased by approximately 8% at the 30 mg/kg/day dose, and by approximately 20% at the 60 mg/kg/day dose, throughout the testing period. Thus, food consumption decreases could account for some, but not all, of the body weight changes observed in the F1 males. It is of interest that decreased body weight in F1 males was observed from day 1 of the study in contrast to P1 males, where changes were not observed until approximately day 36 of dosing.

In female parental rats of the P1 generation, group mean weight gain for the 1-70 day preocohabitation period was decreased by 16% from control at the 60 mg/kg/day dose, with no effect observed at the 30 mg/kg/day dose. Food consumption in P1 female rats was not affected by treatment with pentachlorophenol, in contrast to male rats. Thus, decreases in body weight and weight gain at the 60 mg/kg/day dose level can be supported as a treatment-related effect.

In comparison to male rats pre-mating, the data suggest that female rats were not as sensitive to the effects of pentachlorophenol on body weight and food consumption as males. Although F1 females showed body weight deficits at post-weaning day 1, these deficits did not persist.

Body weight and weight gain in nursing dams was also affected at 60 mg/kg/day pentachlorophenol. In P1 females at 60 mg/kg/day, group mean body weight on day 20 of gestation was decreased 11% from control, and weight gain for the gestation period (days 0-20) decreased 19% from control. In F1 female rats, group mean body weight in gestation day 20 was decreased 22% from control, and weight gain for the 0-20 day gestation period decreased 29% from control. It is noted that the body weight effects in the F1 generation were more significant than those observed in the P1 generation.

The results of estrous cycling analyses in P1 and F1 parental female rats showed no significant treatment related effects. Sperm analyses in P1 male rats also showed no significant treatment related effects. However, effects on sperm morphology ad function were observed at 60 mg/kg/day in F1 male rats. In F1 generation males, the number of sperm observed with a broken flagellum was significantly increased at 60 mg/kg/day (1.5 ± 1.6

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vs 0.6 ± 1.4 in control), and the average testicular spermatid count was significantly decreased at the 30 and 60 mg/kg/day dose levels (157 ± 28.3 and 152.1 ± 32.2 respectively) vs control (175.8 ± 32.5). Testis weight in F1 generation males was decreased at the 30 and 60 mg/kg/day dose levels (1.5667 and 1.3315 grams vs 1.6634 grams in control). The effects of treatment on sperm morphology as related to a reproductive effect of pentachlorophenol are not completely in agreement, as significant sperm effects were not observed in both generations, but significant decreases in litter size were observed. One correlation which may be relevant is the greater decrease in litter size of the second generation, coupled with the more visible effect of pentachlorophenol on sperm parameters in the second generation. However, data from a developmental toxicity study in rats (MRID # 43091702) suggest increases in early resorptions an decreases in litter size at 80 mg/kg/day pentachlorophenol, implying an effect in utero that may be a manifestation of maternal toxicity.

Sexual maturation was affected at 60 mg/kg/day pentachlorophenol. The average day of vaginal patency in F1 females was significantly increased to 36.2 ± 3.0 days at 60 mg/kg/day pentachlorophenol, vs 32.0 ± 0.9 days in control ($p < 0.01$). In fact, days to vaginal patency were significantly increased at all dose levels from control (33.2 ± 1.1 and 33.9 ± 1.8 days at 10 and 30 mg/kg/day pentachlorophenol, respectively). However, according to the report, the values observed at these lower doses were within the historical control range of the performing laboratory, with a reported historical range of 30.1 - 33.7 in 462 control female rats from 16 studies conducted from March 1995 to September 1997.
Preputial separation in F1 male rats was significantly increased at 30 and 60 mg/kg/day pentachlorophenol (46.2±2.1 and 49.6±3.6 days, respectively, vs 44.5±2.0 days in control).

Reproductive performance in both generations was affected at the 60 mg/kg/day pentachlorophenol dose level. In P1 males and females, decreased fertility index was observed, with a slight decrease in number of litters delivered at the 60 mg/kg/day dose level. In F1 males and females, fertility index was also decreased at 60 mg/kg/day, with a significant decrease in number of litters as well as an increased number of intercurrent deaths.

Organ weights were affected in both parental generations at 60 mg/kg/day pentachlorophenol. In P1 males and females, body weight at termination was decreased 13% and 7% respectively. This decrease in body weight was accompanied by a decrease in brain weight in both sexes (4% in males, 8% in females), and increases in liver weight (33% increase in males, 32% increase in females). Kidney weight was also increased in P1 males by 10-11% over

control. In F1 parental rats, body weight for males and females was decreased by 31% and 20% respectively. Brain weights for both sexes was also decreased (11% in males, 9% in females). Liver weight, similar to the P1 generation, was increased 14% in males. Of note is the significant decrease in testes and epididymis weight observed in F1 male rats, not observed in the P1 generation.

Macroscopic evaluations of P1 and F1 parental rats showed an increased incidence of enlarged liver at the 60 mg/kg/day dose of pentachlorophenol. This observation is supported by the significant increase in incidence of several microscopic hepatic lesions in both male and female P1 rats, including centrilobular hepatocyte hypertrophy, inflammation, pigment deposition, and single cell necrosis. These same observations were recorded in the F1 parental generation of rats. The report stated that the hepatocellular centrilobular hypertrophy and vacuolation were expected metabolic effects resulting from oxidative stress, common to several organochlorine compounds. The single cell necrosis and centrilobular pigment observed in increased incidence at 30 and 60 mg/kg/day pentachlorophenol were considered distinct alterations as a result of pentachlorophenol. Also noted at the 60 mg/kg/day dose was bile duct proliferation, only in females. Not observed in the P1 generation but present in the F1 generation male rats at 30 and 60 mg/kg/day pentachlorophenol was focal or multifocal mononuclear inflammatory cell infiltration of the epididymides, and, at 60 mg/kg/day, necrotic germ cells.

For the F1 generation pups, several viability indices were significantly affected at the 60 mg/kg/day pentachlorophenol dose level. Mean litter size, number of live pups, pup deaths days 0-4, viability index, and lactation index were all significantly decreased in relation to lower doses and control values. The same indices were affected in the P1 generation pups, but not to the same degree as in the F1 generation pups. This observation is consistent with the apparent greater effects of high dose pentachlorophenol treatment on F1 parental rats than on P1 parental rats. Organ weights in treated pups were also affected differentially by generation. While no significant organ weight effects were seen in pups of the P1 parents, pups of the F1 parents showed the following: For the F2 male pups reduced weight of the liver (1.19 grams vs 1.77 in control), spleen (0.09g vs 0.18g in control), brain (1.38g vs 1.52 g in control), and thymus (0.1g vs 0.2g in control) were observed at 60 mg/kg/day. At 30 mg/kg/day, significant reductions in brain weight (1.48g) and thymus weight (0.16g) were observed in males. In female F2 pups, a similar pattern of decreased organ weights was observed as that for males. At 60 mg/kg/day, liver weight was reduced (1.14g vs 1.78g in control), as was spleen weight (0.08g vs 0.18g in

control), brain weight (1.33g vs 1.47g in control), and thymus weight (0.1g vs 0.2g in control). Brain weight at 30 mg/kg/day was reduced (1.43g) as was thymus weight (0.16g). The decreases in organ weight for F2 pups as mentioned were in all cases statistically significant.

Based on the data in this study, the Systemic NOEL = 10 mg/kg/day for male and female parental rats. The Systemic LOEL = 30 mg/kg/day for male and female rats, based on decreased body weight and weight gain in F1 generation parental rats, and adverse testicular effects in F1 male rats (decreased testis weight, decreased spermatid count).

The reproductive NOEL = 10 mg/kg/day in this study. The reproductive LOEL = 30 mg/kg/day, based on decreased group mean litter weight.

C. STUDY DEFICIENCIES: Information on homogeneity of the dosing solutions used in this study were not included with this report. Data on homogeneity were presented which represented results of a different study. To be considered acceptable, results on the homogeneit of dosing solutions used in the present study must be submitted.