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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorpicrin: Rat reproduction study. MRID 43391901 and 43391902. D209011. Tox. Chem. No. 214.

TO: Larry Schnaubelt/Susan Jennings (PM 72)
Special Review and Reregistration Division (7508W)

FROM: Stanley B. Gross, PhD, DABT, CIH
Toxicologist/Hygienist
Toxicology Branch I
Health Effects Division (7509C)

Stanley B. Gross 5/14/96

THRU: Joycelyn E. Stewart, PhD
Head, Section II, Toxicology Branch I
Health Effects Division (7509C)

JS 5/15/96

5/15/96

I. SUBMISSION:

The following study was submitted to OPP to fulfill the 2-generation reproduction data requirement for GLN 83-4):

CITATION: James L. Scrardein, MS, ATS. (1994). Two Generation Inhalation Reproduction/Fertility Study in Rats. IRDC, Mattawana, MI. #656-011. September 28, 1994. MRID NO.: 43391901 (Range-finding); NO. 43391902 (Main study).

SPONSOR: Chloropicrin Manufacturer's Task Force, c/o Niklor Chemical Co. , Long Beach, CA.

II. CONCLUSION:

The study is ACCEPTABLE. The DER for the study is attached.

III. STUDY SUMMARY.

In a two generation reproduction study (MRID 433919-02), Chloropicrin (CP) 99% composite was administered in whole body

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exposure chambers to Charles River Crl:CD VAF/Plus rats, 26 animals/sex/dosage group each for F0 and F1 generations. The purity of the CP composite was assumed to be 99% based on previous communications with the sponsor. The exposure concentrations of 0 for control group (CG), 0.5 ppm for the low dose group (LDG), 1.0 ppm for the mid-dose group (MDG) and 1.5 ppm for the high dose group (HDG) and was administered 6 hours/day and 7 days/week during all exposure periods.

The CP exposures in the present study to F0 parents began at age 43 days and lasted for 76 days. Premating exposures to F1 adults began at age of 28 days and lasted for 83 days. Exposures to males continued to the end of gestation. Exposure of adult females were interrupted during the 2-4 week mating periods and the three week lactation periods.

Administration of chloropicrin to male and female rats produced no toxicity to F0 and F1 parents or to any of the offspring (the F1 and F2 generations) based on clinical signs, body weight, reproductive performance, litter/pup growth and morphology. The investigators cited minimal pulmonary inflammation in the high-dose F0 females as high dose toxicity; however, low level inflammatory changes in the lungs were seen uniformly throughout all treatment group adults necropsied. Therefore Toxicology Branch I does not consider the lung inflammation in the high dose females to be biologically significant.

Conclusion. The NOEL for this study is considered to be 1.5 ppm, the highest dose tested. The study is considered ACCEPTABLE.

Special Review Criteria (40 CFR 154.7): None

CP89011.MMO. May 14, 1996 E1 SBG.

2

EPA Reviewer: Stanley B. Gross, PhD , Date 2/29/96
Review Section, Toxicology Branch (7509C)
EPA Section Head: Joycelyn E. Stewart. PhD , Date 5/15/96
Review Section _, Toxicology Branch __ (7509C)

DATA EVALUATION RECORD

***I STUDY ID**

STUDY TYPE: Multigeneration Reproduction Study - Rat. OPPTS 870.3800 (OPP GLN 83-4)

DP BARCODE: D209011

TOX. CHEM. NO.: 214 SUBMISSION CODE: S476359

P.C. CODE: 081501

TEST MATERIAL: Chloropicrin (Composite)

CITATION: James L. Scrardein, MS, ATS. (1994). Two Generation Inhalation Reproduction/Fertility Study in Rats. IRDC, Mattawana, MI. #656-011. September 28, 1994. MRID NO.: 43391901 (Range-finding study); NO. 43391902 (Main study).

SPONSOR: Chloropicrin Manufacturer's Task Force, c/o Niklor Chemical Co. , Long Beach, CA.

COMPLIANCE:: Signed and dated GLP, confidentiality and Quality Assurance statements were provided.

***II. EXECUTIVE SUMMARY**

In a two generation reproduction study (MRID 433919-01), Chloropicrin (CP) 99% composite was administered in whole body exposure chambers to Charles River Crl:CD VAF/Plus rats, 26 animals/sex/dosage group each for F0 and F1 generations. The purity of the CP composite was assumed to be 99% based on previous communications with the sponsor. The exposure concentrations of 0 for control group (CG), 0.5 ppm for the low dose group (LDG), 1.0 ppm for the mid-dose group (MDG) and 1.5 ppm for the high dose group (HDG) and was administered 6 hours/day and 7 days/week during all exposure periods.

The CP exposures in the present study to FO parents began at age 43 days and lasted for 76 days. Premating exposures to F1 adults began at age of 28 days and lasted for 83 days. Exposures to males continued to the end of gestation. Exposure of adult females were interrupted during the 2-4 week mating periods and the three week lactation periods.

Administration of chloropicrin to male and female rats

produced no toxicity to F0 and F1 parents or to any of the offspring (the F1 and F2 generations) based on clinical signs, body weight,-reproductive performance, litter/pup growth and morphology. The investigators cited minimal pulmonary inflammation in the high-dose F0 females as high dose toxicity; however, low level inflammatory changes in the lungs were seen uniformly throughout all treatment group adults necropsied. Therefore Toxicology Branch I does not consider the lung inflammation in the high dose females to be biologically significant.

Conclusion. The NOEL for this study is considered to be 1.5 ppm, the highest dose tested. The study is considered ACCEPTABLE.

Special Review Criteria (40 CFR 154.7) None

***III. STUDY DESIGN**

A. EXPERIMENTAL TREATMENT GROUPS.

The adult animals from both F0 and F1 generations were randomly assigned to the test groups shown in Table 1.

*T1 TABLE 1 Parental Animal Assignments for the **F0 and F1 Generations.**

Test Group	Experimental groups. (ppm)	Animals/group	
		Males	Females
Control (CG)	0	26	26
Low (LDG)	0.5	26	26
Mid (MDG)	1.0	26	26
High (HDG)	1.5	26	26

Exposure Schedules. The females adult animals were exposed to CP in whole body exposure chambers 6 hours/ day, 7 days per week during premating periods, predelivery times (gestation days 0 to 20) and during post-weaning periods. Males were exposed through premating and gestation periods. The premating period for the F0 generation parents began at the age of 43 days and continued for 76 days (study weeks 0-11). The premating period for the F1 generation parents began at age 28 and continued for 83 days. Mothers with litters from both F0 and F1 generations were removed from their litters in plastic cages for 6 hour exposures on lactation days 5 to 21.

Group Assignments The first generation of parental animals

(F0 adults) were randomly assigned by computer to their experimental groups from an initial group of animals whose body weights were within ±1.5 SD of the overall mean. The selection of pups from F1 offspring to form the F1 adult animals at gestation day 4 (GD4) was also done by computer randomization. Selections of F1 weanlings (26/sex/group) on lactation day 21 (LD21) to become F1 parents were also made by computer random selections from each of the litters from within an experimental group.

B. DOSE SELECTION RATIONALE.

Dose selection was based on the results of two studies: a pilot reproduction study and a recent 90 day inhalation study in rats, neither of which were cited by reference nor discussed in adequate detail. The pilot study involved 6 weeks of daily exposures to 0.4, 1.0 and 2.0 ppm of CP. At the high dose both males and females experienced decreased body weights, food consumption and decreased litter sizes and decreased uterine implantations. In the 90 day inhalation study, rats were exposed to 0.3, 1.0 and 3.0 ppm of CP, 6 hours/day, 5 days per week for 13 weeks. The 3.0 ppm exposure animals experienced a 30% mortality, decreased body weights, reduced food consumption and adverse hematological and clinical chemistry effects. The 1.0 ppm treatment groups experienced decreases in body weights and increased lung weights associated with pulmonary irritation.

Based on these two studies, the treatment levels of 1.0 ppm (MDG) and 1.5 ppm chosen for the high-dose group for the present study.

D. HISTORICAL CONTROLS.

Appendix S of the study report included historical control data from 140 studies identified only by number and date, from March 1988 to October 1992, were included. Substantial blocks of data were missing for many of the studies listed.

E. STATISTICAL METHODS.

All statistical tests were performed on a VAX computer using SAS statistical and in-house software. Analyses of variance were used to analyze differences for parental body weight and food consumption, maternal body weight changes during gestation and lactation, copulatory intervals, organ weights, mean numbers of still born and live born pups per litter and mean pup body weights. Bartlett's (test for homogeneity of variance) and/or Dunnett's test (for pair-wise comparison) were used when ANOVs were not significant. Fisher's exact test by Siegel was

used for male and female fertility and copulatory indices and the pregnancy indices. Kruskal-Wallis and/or Mann-Whitney with Bonferroni corrections were used for pup survival indices.

***IV. MATERIALS**

A. TEST MATERIAL:

Chloropicrin, purity not discussed but was assumed to be 99%+ a.i. composite based on previous communications with the Chlorpicrin Task Force.

Description: referred to as a"Clear liquid". Any comments to a strong odor and irritable vapors were missing.

Lot/Batch #: 920130-1. Obtained from Niklor Chemical Co. Stability of diluted concentrations are reported in text below.

B. TEST ANIMALS

Species: Rats. Strain: Charles River Charles River Crl:CD VAF/Plus rats.

Source: Charles River Laboratories, Inc, Portage MI. Age and weight at study initiation: approximately 35 days.

Weights at the beginning of the dosing, males 165 to 132 gm and females, 137 to 161 gm .

Husbandry: Housing -- Individual cages except when kept in exposure chambers. Maintained under standard laboratory conditions of temperatures, 72°±2.6°F, and relative humidity of 58 ±2.6%; 12 hour light cycle.

Acclimation period: 8 days.

Diet - Animals were fed Certified Rodent Chow #5002 (Purina Mills, Inc., St. Louis) and tap water ad libitum except when exposed to CP in the exposure chambers.

***V. METHODS AND PROCEDURES**

A. EXPOSURE METHODS.

Chambers: Four rectangular 16 cu.m. chambers made of stainless steel and glass equipped with a tangential intake plenum were used for animal exposures. Chamber intake air was obtained from room air filtered through HVAC filters. The temperature and humidity within the chambers were assumed to be the same as the animals rooms housing the chambers. The animals were kept in the chambers 24 hours per day during the exposure periods and were removed briefly just prior to and after the exposures in order to clean the chamber.

Generation System. CP used in the chambers was first pre-

diluted with air in Tedlar bags (Anspec Co.) housed within a plexiglass box (CVM Bag Cart). The CP in these bags was referred to as Concentrated Vapor Mixtures or CVM. FMI Pumps (Fluid Metering Inc.), were used to feed the CVM CP air mixture into the chamber intake air stream. The CVMs mixtures were prepared weekly and daily for 7 days to supply the CP used in the experimental exposure chambers. The stability of CVM mixtures in the Tedlar bags was evaluated by sampling the diluted CP at 0.2, 1.7 and 3.9 ppm daily for 7 days.

Concentrations. The concentrations of CP in the exposure chambers were monitored on-line using infrared monitors (Wilkes MIRAN) from sampling from closed loop recycling system. The nominal concentrations were determined for each run by calculating the amount fed into the air stream divided by the air flow for the exposure time. Analytical concentrations in the chambers were determined from samples taken at the breathing zone of the animals using a computerized automatic sampling system (Hewlett-Packard Model 3396 Series II0. CP in the chamber samples were analyzed using Gas chromatographic Model Varian 3700 (GC) and electron capture detector (ECD). Micro liters of CP (6 to 36 uL) were diluted in air to serve as calibration of 0.3, 0.80, 1.3 and 1.8 ppm) concentrations for the determination of calibration curves. .

Aerosol Contamination. A check against contaminating aerosols was done using a light scattering aerosol sensor (Sibata Digital Dust Indicator P5) which detected aerosol concentrations down to 0.38 ug/L.

Chamber Distributions. Chamber distributions were validated by quadruplicate sampling from within the chambers at 4 points distributed around the front to back and top to bottom of the animals cages.

B. MATING PROCEDURES.

One male was caged with one female from the same test group until evidence of copulation was observed. Evidence of copulation was based on finding a sperm plug in the female's vagina, on the pan in the cage or finding sperm taken from the vagina. If there was no evidence of mating after 7 days of pairing with the first male, the first male was replaced with a second male for 7 additional days. If a third male was not able to mate with a female, the female was euthanized and examined for genital defects. Similarly, males that would not mate were euthanized and examined microscopically for sperm. Spermatogenesis was evaluated by microscopic inspection of the epididymis.

Gestation day zero was designated when there was evidence of

copulation and the female transferred back to wire cages for continued CP exposures. On gestation day 20, the females were transferred to plastic cages with wood chip bedding for birthing and lactation periods.

C. PARENTAL OBSERVATIONS.

Toxicity. All animals were observed twice daily for signs of toxicity. Male and female body weights and food consumption were measured weekly prior to mating. Females were weighed on gestation days 0, 6, 15 and 20 and on lactation days 0, 7, 14 and 21.

Reproductive performance. Reproductive parameters measured included numbers of adults paired, mated, time to mating, fertile males and females, gestation periods and uterine implantations. Spermatogenesis by histopathological examination was determined in the non-fertile males. The following indices were calculated:

$$\text{Male fertility index} = \frac{\text{No. females impregnated}}{\text{No. males mated}}$$

$$\text{Female fertility index} = \frac{\text{No. females pregnant}}{\text{No. females mated}}$$

$$\text{Copulatory index} = \frac{\text{No. mated (male or female)}}{\text{No. paired}}$$

Adult Necropsy observations: Non-mating males and females were euthanized and necropsied for organ pathology listed below. All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to post mortem examinations (macroscopic and or microscopic examination).

The following tissues (X) were prepared for microscopic examination and weighed (XX):

X	Ovaries	X	Epididymides
X	Uterus	X	Prostate
X	Vagina/cervix	X	Seminal vesicles
X	Lesions	XX	Testes

D. LITTER AND PUP OBSERVATIONS:

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born dead or found dead. The following data were recorded for each litter from the F1 and F2 offspring:

--Number of live and dead pups, pup weight, sex, and external abnormalities at birth
--Dead and/or abnormal pups daily
--Pup weight on days 0, 4, 7, 14 and 21
--Selection was made by random numbers using the same data generated for the standardization of litter size on day 4 post partum.
-- Litter data obtained were the number of litters produced, mean litter pup weights and numbers and deformed pups.

The following F1 indices were calculated:

$$\text{Live born index} = \frac{\text{No. live pups born}}{\text{No. live + dead pups born}} \times 100$$

$$\text{Viability index} = \frac{\text{No. live pups at day 4}}{\text{No. pups born alive}} \times 100$$

$$\text{Lactation index} = \frac{\text{No. live pups at day 22}}{\text{No. pups born alive}} \times 100$$

Standardization of F1 and F2 Litters. On gestation day 4, each litter was reduced to 8 (generally 4 male and 4 female pups) using random selection generated by a computer. The unselected pups were carefully examined grossly and discarded. On lactation day 21, 26 males and 26 females from the F1 offspring were randomly selected to become the F1 parents which were mated after the pre-mating period of exposure time. The non-chosen pups from the F1 offspring were examined and discarded.

***VI. RESULTS.**

A. EXPOSURE CONDITIONS.

Mean chamber concentrations for each test group are shown in Table 2. These data show that concentrations sought were achieved.

9

*T2 Table 2. Chamber Exposure Concentrations (ppm).

	Experimental Groups		
	LDT	MDT	HDT
F0 Generation			
Nominal Concns Actual Concns.	0.47 0.46±0.011	0.91 0.97±0.033	1.6 1.5±0.054
F1 Generation			
Nominal Concns Actual Concns.	0.47 0.45±0.039	0.88 0.96±0.021	1.6 1.5±0.0

Stability analyses of CP the CVM in the Tedlar bags resulted in samples which ranged from 93% to 105% of the original concentration without any decrement over time. Chamber distribution analyses of four chamber sites for three concentration levels of 0.2, 1.7 and 3.9 ppm were from 93% to 107% of the target concentrations. Aerosol analyses of chamber atmospheres indicated that particulate materials were below 0.38 ugm/L, the limit of detection for the aerosol sensor.

B. PARENTAL OBSERVATIONS.

Selected parental data for F0 and F1 generations are presented in Tables 3 and 4.

Mortality. Mortality data (Tables 3 and 4) in adult animals were dispersed throughout most of the exposure groups at low incidence (generally zero or 1/group) without any relationship to CP administration. There were no deaths of parents in the F1 generation. Deaths in the F0 generations included one male in the mid-exposure group, one female each in the control, low-dose and mid-exposures groups and two males and two females in the high-exposure groups died prior to scheduled sacrifice. Four F0 males died/euthanized in extremis, one each in the low and mid dose groups and two in the high dose groups. One of the high dose group males was sacrificed in extremis secondarily to a fractured femur.

Clinical Signs: There were no clinical signs of toxicity due to the exposures to CP. Fifty to 77% of the males and 62 to 88% of the females of the F0 generation showed no visible abnormalities. Antemortem observations were of low incidence and included various unremarkable observations, such as malocclusions,

*T3 TABLE 3. Selected Parental Data, F0 Generation.

Observation	Dose Group			
	Control	Low	Mid	High
F0 generation - males				
Mortality M/F	0/26	0/26	1/26	2/26
Mean Body Weight Week 0 Week 10 Gain Wks 0-17	 176 288 480	 180 291 491	 180 283 494	 182 286 493
Mean Food Consumption (gm/animal/day) Weeks 10	18.8	19.7	19.0	19.0
Relative Testicular Wt. (%) No. Animals	7.52% 9	7.51 4	6.74 2	6.39 8
F0 generation - females				
Mortality	1/26	1/26	1/26	2/26
Mean Body Weights (gm) Week 0 Week 10 Wt. gain (0 to 10 wks)	 149 288 139	 150 291 141	 148 283 135	 150 286 136
Mean Gestation BW gain (gm) Days 0-21	135	146	144	128
Mean Lactation BW gain (gm) Days 0-21	21	22	15	23
Mean Food Consumption (gm/animal/day) Week 10 Gestation 0-20 days Lactation 0-21 days	18.8 23.9 45.5	19.7 24.0 44.6	19.0 22.9 45.0	19.0 22.1 45.1

*T4. TABLE 4. Selected Parental Data, F1 Generation

Observation	Dose Group			
	Control	Low	Mid	High
F1 generation - males				
Mortality D/L	0/26	0/26	0/26	0/26
Body Weight				
Week 4	99	97	92	96
Week 10	409	409	390	392
Gain Wk 4-24	483	497	482	468
Food Consumption (gm/animal/day) Weeks 10	26.9	27.4	25.8	27.3
Relative Testicular Wt (%)	6.40	6.43	6.64	6.39
No. Animals	8	9	6	8
F1 generation - females				
Mortality	0/26	0/26	0/26	0/26
Body Weights (gm)				
Week 4	92	87	85	85
Week 10	252	252	241	238*
Wt. gain (4 to 10 wks)	160	165	160	153
Body Weight gain (gm)				
Gestation days 0-20	136	152	140	128
Lactation days 0-21	8	12	16	23
Food Consumption (g/animal/day)				
Weeks 0-10	18.8	19.7	19.0	19.0
Gestation days 0-20	23.9	24.0	22.9	22.1
Lactation day 0-21	45.5	44.6	45.0	45.1

* Significantly different from the control group; $p<0.05$.

materials around the eyes and hair loss. The antemortem observations of the F1 adult males and female were similar to the F0 adults, showing no abnormalities that were compound related.

Body Weight Changes. Body weights changes (Tables 3 and 4) in the F0 males and females were comparable to controls. Body weight changes in the F1 generation adults were intermittently different from control animals but were not dose related to the CP exposure concentrations.

Food Consumption. Food consumption (Table 3 and 4) on the parents of both generations were unaffected by the test article exposure. Changes in F0 of the F1 generations were intermittently different from controls but not dose related.

Reproductive Parameters F0 and F1 Adults. Selected reproductive performance data for the F0 and F1 adults are presented in Tables 5 and 6. There were no findings that were relatable to the exposures to CP. Male and female copulatory indices were comparably high in both the F0 and F1 adults, however the fertility indices were variable across the different experimental groups being generally low in the control and high dose groups and somewhat higher in the low and mid dose groups. Evaluation of the F0 male and female fertility indices and the pregnancy indexes was difficult due to the low values noted in the control group. The mid dose group fertility values were significantly increased in the F0 adults over the controls.

B. OBSERVATIONS FOR THE F0 AND F1 OFFSPRING.

Selected litter/pup data for the F1 and F2 generation offsprings are provided in Tables 7 and 8. Except for the F1 control group, the numbers of litters, the numbers of pups born alive and dead, the litter sizes and general pup performance were comparable for both the F1 and F2 generation offspring. None of the parameters for litter size, pup numbers and growth were significantly different and were considered to be within biological variability. Sex ratios for new born pups within litters were not presented in the report.

Morphological Findings for Offspring. There were no findings in the offspring of the F1 or F2 offspring indicating any effects associated with CP exposure.

*T5 TABLE 5 Selected Reproductive Performance, F0 Adults

OBSERVATION	DOSE GROUP (ppm)			
	Control	LDT	MDT	HDT
F0 Generation Adults				
Median precoital interval (days)	2.5	2.5	2.5	3.8
MALES				
Mated	25	25	25	22
Fertile Percent	57.7	80.0	88.5	69.6
Copulatory Index/ Percent	25/26 96.2	24/25 96.0	25/26 96.2	22/23[a] 95.7
Male Fertility No./ Percent	15/26 57.7	20/25 80.0	23/26 88.5[1]	16/23[a] 69.6
FEMALES				
Number mated	26	25	26	24
Copulatory Index (mated/paired) Percent	25/26 96.2	25/25 100	25/26 88.5	24/24[b] 100
Fertility Index (litters/paired) Percent	15/26 57.7	21/25 84.0	23/26 96.2	17/24[b] 70.8
Pregnancy Index . litters/mated) Percent	15/25 60.0	21/25 84.0	23/26 88.5[1]	17/24[b] 70.8
Median gestation interval (days)	22.1	22.4	22.3	22.0
Mean implantations sites.	15.1	15.0	15.2	13.9
Mean implantation losses (post-implantation losses minus pups born.	1.3	1.7	0.9	1.1

[a] Does not include one male paired with a female which died prior to mating. [b] Does not include one female which died prior to mating. [1] Statistically significantly different from control, $p<0.05$.

*T6 TABLE 6 Selected Reproductive Performance, F1 Adults (F2 Mating).

OBSERVATION	DOSE GROUP (ppm)			
	Control	LDT	MDT	HDT
F1 Generation Adults				
Median precoital interval (days)	3.9	3.7	3.0	4.9
MALES				
Mated	22	24	25	22
Fertile	18	18	20	19
Copulatory Index (mated/paired) Percent	22/26 84.6%	24/26 92.3%	25/26 96.2	22/26 84.6%
Fertility index (litters/paired) Percent	18/26 69.2%	18/26 69.2%	20/26 76.9%	19/26 73.1%
FEMALES				
Number mated	25	26	26	26
Number fertile	19	18	20	20
Gestation length (days)	22.2	22.3	22.5	22.3
Copulatory Index (mated/paired) Percent	25/26 96.2%	26/26 100%	26/26 100%	26/26 100%
Fertility Index (litters/paired) Percent	19/26 73.1	18/26 69.2	20/26 76.9	20/26 77.6
Pregnancy Index (litters/mated) Percent	19/25 76.0%	18/26 69.2%	20/26 76.9%	20/26 76.9%
Mean implantation losses (implantations less live pup)	0.8	0.8	1.2	1.2

F1 Offspring: During the F1 lactation period 9,4,3,3 pups were missing (days 1-11) and were assumed to be cannibalized. On lactation days 12 and 15, respectively, 7 and 8 pups in the control and mid-exposure groups were euthanized due to the death of the respective dams. One pup in the high dose group died on lactation day 21 due to non-test article related mechanical injury.

Antemortem observations for the pups from all experimental groups were essentially normal with aberrations at low incidence. Abnormal findings were generally restricted to deformities of the tail, skin lesions and low body weight.

Pups euthanized because of dam deaths, those necropsied in culling process and those examined at weanling selection

*T7 TABLE 7 Selected Litter/Pup Data, F1 Offspring.

OBSERVATION	DOSE GROUP (ppm)			
	Control	LDT	MDT	HDT
F1 Generation Pups/litters				
No. of Litters	15	21	23	17
Pups Born (dead and alive)	207	279	328	220
Pups born live	202	275	320	215
Live Pup Index (%) at birth	97.6	98.6	97.6	97.7
Viability Index (% live pups on day 4 vs live pups at birth)	95.0	98.5	98.4	98.1
Lactation Index (live birth/day 21%)	55.4	58.2	52.8	60.4
Mean litter size (Day 1)	13.8	14.0	14.3	12.9
Mean pup weight (g)(Day 1)	6.5	6.8	6.6	6.8
Mean pup weight (gm) Day 4 Before culling After culling	 11.0 11.0	 11.1 11.1	 10.2 10.3	 10.9 10.9
Mean pup weight (g)(Day 21) males/females	50.3/ 47.6	48.7/ 45.8	46.8/ 44.9	47.2/ 46.1

indicated a number of expected morphological abnormalities including hydronephrosis, absent innominate vessel, distended ureters, and other abnormalities which were not associated with dosing with CP.

F2 Offspring: During the F2 lactation period, a total of 24 pups from two litter from the control groups were euthanized on days 3 and 9 due to removal from the study room and potential exposures to other test articles. Five, 13, 7 and 11 pups were lost from the control, low, mid and high dose groups assumed to be cannibalized.

Antemortem findings (tail abnormalities, emaciation, inguinal laceration, pale color, and the like) were noted but not relatable to CP exposures. Likewise, necropsy finding (hydronephrosis, cardiac and liver abnormalities) were of low incidence and not relatable to CP exposures.

*T8 TABLE 8 Selected Litter/Pup Data, F2 Offspring

OBSERVATION	DOSE GROUP (ppm)			
	Control	LDT	MDT	HDT
F2 Generation Offspring				
Number of litter	19	18	20	20
Pups born (live plus dead)	254	242	278	270
Pups born alive Live birth index (%)	250 98.4%	239 98.8%	278 100%	260 96.3%
Pups born dead	4	3	0	10
Mean litter size (Day 1)	13.4	14.3	13.9	13.5
Percent malformed pup born				
Viability Index (Birth to day 4)	91.5	95.8	98.2	95.8
Lactation index (pups live 21 days/live births)	52.8	55.0	55.4	57.3
Mean pup weight (gm) day 1	6.3	6.7	7.0*	6.5
Mean pup weight (gm) day 4 Before culling After culling	 10.1 10.2	 10.4 10.5	 11.3 11.5	 10.7 10.7
Mean pup wt. (gm) day 21 Males/Females	47.7/ 46.5	52.1/ 47.3	48.8 47.0	46.8 44.2

C. NECROPSY FINDINGS ADULTS

Gross Pathology: Most 19-23 of the males from each group of F0 and F1 adults were considered to be within normal limits. Gross abnormalities in these animals were limited to skin (alopecia primarily) lymph nodes and minimal pulmonary findings. Somewhat fewer of the F0 and F1 females (12-19/per group) were considered normal. The abnormal findings again were confined primary to the skin and lung (discoloration). None of the finding for the adults were related to exposures to CP.

Organ Weights. Only the weights of the testes of the F0 and F1 males were measured. The summary of these data (relative testicular weights) are shown with the adult data shown in Tables 3 and 4. Only 2 to 9 males per group (of 26/group mated) were weighed. There were no statistically significant variations in mean absolute body weight, absolute testis weights or relative testis/body weights for the F0 or F1 males.

Spermatogenesis. According to the report (page 26), spermatogenesis evaluation for 9,4,2 and 8 F0 males from the control, low, mid- and high-dose groups, respectively, were examined for sperm present, motility and morphology. One animal in the high dose group showed nonmotile sperm. The report did not explain how these measurements were obtained other than the histopathological examination of the epididymis.

Histopathology: The histopathological assessment of the organ pathology was focused on the sex organs listed above under necropsy procedures. Lesions of other organs were also prepared for histologic examination however, these numbers of organs examined were limited as to the number of animals and to the number of animals have only 0 to 1 lesions per animal.

In their analyses of histopathology data, the report investigators focused primarily on acute/subacute inflammatory lesions in the lung that appeared to be test-article limited to the lungs of F0 females as shown in Table 9. These findings were not statistically significant and were one of several other pulmonary findings that were distributed within all treatment groups, showing essentially no relationship to CP administration. All animals exhibited trace levels (7-10 per 26-25 in groups) without dose-response patterns. Table 9 shows a number of these finding in the lungs of the F0 and F1 adult animals.

*VII. DISCUSSION

A. EXPERIMENTAL DESIGN.

The experimental design for this study is essentially acceptable, however certain information was either poorly presented or lacking. The distribution of sexes in the new born pups was not included in the data. Purity of CP was not discussed, and it should also be assumed that CP had an odor and was irritating to the eye and nose (unless exposure to it was well controlled. The odor threshold to CP is 0.3 ppm (OSHA documentation for the PEL). It would also be helpful if the ranges of the various parameters of the historical control data were summarized.

Exposure Questions. Because there was little toxicity observed (discussed below) in the parents and pup, there is a question of whether there was adequate distribution of the CP within the chamber or whether the concentration of CP was sufficiently high to produce the overt toxicity required by the reproduction guideline. The design of the chambers, the location of the sampling areas especially with regard to the distribution studies, should have been discussed in more detail.

*T9 Table 9. Selected Lung Histopathology Findings.

	DOSE GROUP (ppm)			
	Control	LDG	MDG	HDG
F0 Generation MALES				
No. Animals	26	0	1	24
Hemorrhage, mild to moderate	0	0	1	1
Infiltration, lymphoid, peribronchial, trace/mild	26	0	1	24
F0 Generation FEMALES				
No. Animals	15	20	23	17
Hemorrhage, trace to moderate	4	4	5	5
Infiltration, lymphoid, peribronchial, trace	15	20	23	17
F1 Generation MALES				
No. Animals	26	3	1	26
Hemorrhage, trace/mild	1	0	0	2
Infiltration, lymphoid, peribronchial, trace	26	1	0	26
F Generation FEMALES				
No. Animals	26	26	26	26
Hemorrhage, trace/mild	1	2	4	4
Infiltration, lymphoid, mild	26	26	26	26
Inflammation, Trace Mild	 7 0	 9 1	 10 2	 7 4

Systemic Toxicity: The authors indicated that the toxicity of the high dose animals was based on pulmonary inflammation seen only in the females of the F1 adults. Their data are shown in the bottom of Table 9 as 0, 1, 2 and 4 animals out of the control, low-, mid- and high-dose groups respectively. These numbers were not found to be significant. The levels of inflammation designated as mild were 7, 9, 10 and 7, respectively which does not support the conclusion of a dose response. Also included in Table 9 are the data for pulmonary hemorrhage and peribronchial lymphoid infiltration. Hemorrhages in the lung could be due to the irritation of CP but the hemorrhages are distributed evenly between the experimental exposure groups. Lymphoid infiltration generally associated with chronic inflammation was seen throughout all the treatment groups without reference to dosing. An examination of other histological findings reported also do not suggest that the lung was effected by the CP administration.

Reproductive Toxicity: Reproductive toxicity either in the adults or in the offspring were absent in this study. CP is quite toxic and is irritating to the mucous membranes at low concentrations. Since the pilot study reported toxic effects at 2 ppm, this is justification for the high dose in this study being set at 1.5 ppm.

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