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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Methylene bis (thiocyanate): Review of Toxicological Data (83-4)

MRID No.:	420286-01
PC No.:	068102
ID No.:	0608102-001448
HED Project No.:	2-0125
Caswell No.:	565
Submission No.:	S404646
DP Bar Code No.:	D169619

TO: B. Briscoe/B. Crompton, PM # 51
Registration Division (H7505C)

for THRU: Roger Gardner, Section Head Pamela M. Hurley 7/13/93
Review Section 1
Toxicology Branch
Health Effects Division (H7509C)

FROM: Nguyen Bich Thoa, Ph.D 09/26/92
Review Section 1
Toxicology Branch I
Health Effects Division (H7509C) KB 7/20/93

Registrant: Buckman Laboratories Inc.
Memphis, TN.

ACTIONS REQUESTED:

Toxicological Data Review of a reproduction study in rats (83-4) entitled: Methylene bis (Thiocyanate) (MTC) - Two-Generation Reproduction Study in Rats (MRID No. 420286-01).

CONCLUSIONS:

The reproductive toxicity potential of Methylene bis (thiocyanate), a.k.a. MTC, was studied by dosing SD (CD) rats, by oral gavage, through 2 generations (F0-F1; F1-F2), with one mating period per generation. The dose range used was 0, 1.0, 2.5, and initially 5.0 mg/kg/day. The initial high dose caused 4 F0 rats (1 male and 3 females) to be sacrificed in extremis

1

within the first 16 days of dosing, and was reduced to 4.0 mg/kg/day (definitive high dose) from dosing day 17 to the end of the study.

The NOEL for parental systemic effect was 1.0 mg/kg/day (LD) and the LOEL was 2.5 mg/kg/day (MD), based on one F1 (female) mortality associated with necropsy findings of caecum distention with gas. The initial high dose (5 mg/kg/day) caused 4 deaths (See above), most or all of which were preceeded by adverse clinical signs of breathing difficulty (4/4), piloerection (4/4), and swollen abdomen (3/4), and were associated with necropsy findings of gastrointestinal distention by gas (3/4 intestinal distention and 1/4 caecum distention). The definitive high dose (4 mg/kg/day) was also associated with one F1 (male) death. Some of the above described clinical signs were also observed in a few surviving mid-dose and high dose F0 rats (Breathing difficulty: 1 MD and 3 HD; piloerection: 2 MD and 2 HD; swollen abdomen: 1 HD).

MTC 1-4 mg/kg/day did not adversely affect body weight, body weight gain, feed consumption, or any reproductive parameter (precoital interval, oestrus stage, male/female fertility indexes, duration of gestation, gestation index, number of implants/pregnancy, number of pups born dead or alive per dam) in either the F0 or F1 parental groups.

MTC 1-4 mg/kg/day did not adversely affect any of the litter parameters (number of live pups/litter at lactation day 0, 4, 14, or 21, birth, live birth, viability, lactation, and overall survival indexes, male/female pups body weight, and litter weight during the entire lactation period) in either the F1 or F2 litter groups. The NOEL for reproductive effect was 4.0 mg/kg/day (HD).

Core Classification: This study has minimally satisfied the toxicological data requirements for a 2-generation reproductive toxicity study in rats (83-4) and is core classified Minimum (See reviewer discussion Section of attached DER for a list of deficiencies/deviations from acceptance criteria).

A DER on the above referenced study is attached.

Primary Review by: Nguyen B. Thoa, Ph.D. 09/26/92
Review Section 1, Toxicology Branch I/HED
for Secondary Review by: Roger Gardner Pamela M. Hurley 7/13/93
Section Head, Review Section 1, Toxicology Branch I/HED

DATA EVALUATION RECORD

STUDY TYPE: Two-Generation Reproduction Study in Rats (83-4)

IDENTIFICATION Nos:

PC No:	068102
ID No:	0608102-001448
HED Project No.:	2-0125
EPA MRID No.:	420286-01
Caswell No.:	565
Submission No.:	S404646
DP Barcode No:	D169619

TEST MATERIAL: Methylene bis (thiocyanate) Technical; 99.3% a.i.

SYNONY MS: MTC; MBT

SPONSOR: Buckman Laboratories Inc., Memphis, TN.

STUDY NUMBER: IRI Project No. 436385; Report No. 7374.

TESTING FACILITY: Inveresk Res. International, Scotland.

TITLE OF REPORT: Methylene Bis (Thiocyanate) (MTC) - Two generation Reproduction Study in Rats.

AUTHORS: J. A. Wilson and S. J. Barton

DATE REPORT ISSUED: October 30, 1990

CONCLUSIONS: The reproductive toxicity potential of Methylene bis (thiocyanate), a.k.a. MTC, was studied by dosing SD (CD) rats, by oral gavage, through 2 generations (F0-F1; F1-F2), with one mating period per generation. The dose range used was 0, 1.0, 2.5, and initially 5.0 mg/kg/day. The initial high dose caused 4 F0 rats (1 male and 3 females) to be sacrificed in extremis within the first 16 days of dosing, and was reduced to 4.0 mg/kg/day (definitive high dose) from dosing day 17 to the end of the study.

The NOEL for parental systemic effect was 1.0 mg/kg/day (LD) and the LOEL was 2.5 mg/kg/day (MD), based on one F1 (female) mortality associated with necropsy findings of caecum distention with gas. The initial high dose (5 mg/kg/day) caused 4 deaths (See above), most or all of which were preceeded by adverse clinical signs of breathing difficulty (4/4), piloerection (4/4), and swollen abdomen (3/4), and were associated with necropsy findings of

gastrointestinal distention by gas (3/4 intestinal distention and 1/4 caecum distention). The definitive high dose (4 mg/kg/day) was also associated with one F1 (male) death. Some of the above described clinical signs were also observed in a few surviving mid-dose and high dose F0 rats (Breathing difficulty: 1 MD and 3 HD; piloerection: 2 MD and 2 HD; swollen abdomen: 1 HD).

MTC 1-4 mg/kg/day did not adversely affect body weight, body weight gain, feed consumption, or any reproductive parameter (precoital interval, oestrus stage, male/female fertility indexes, duration of gestation, gestation index, number of implants/pregnancy, number of pups born dead or alive per dam) in either the F0 or F1 parental groups.

MTC 1-4 mg/kg/day did not adversely affect any of the litter parameters (number of live pups/litter at lactation day 0, 4, 14, or 21, birth, live birth, viability, lactation, and overall survival indexes, male/female pups body weight, and litter weight during the entire lactation period) in either the F1 or F2 litter groups. The NOEL for reproductive effect was 4.0 mg/kg/day (HD).

Core Classification: This study has minimally satisfied the toxicological data requirements for a 2-generation reproductive toxicity study in rats (83-4) and is core classified Minimum (See Reviewer Discussion Section for a list of deficiencies/deviations from acceptance criteria).

A. MATERIALS

Test Compound: Purity: 99.3% a.i.; Description: A brown crystalline solid (micronized before use); Batch No: 7-0846; Storage: At room temperature, in the dark; Route of administration: Oral gavage.

Vehicle: 0.5% v/v high viscosity carboxymethyl cellulose salt in water; Dose-volume: 5 ml/kg body weight.

Test Animal: Species: Rats; Strain: SD (CD); Source: Charles River Labs., Kent, U.K.; Age: Four weeks old at receipt (02/01/1990); Weight at receipt: 60-110 g; Acclimation Period: 10 days.

Environment: 20 ± 2°C; 55% ± 10 rel. humidity; 12-hr light/12-hr dark (7am-7pm); Housing: Two of same sex/cage during pre-mating period; 1 male and 1 female/cage during mating period; 1 female with litter/cage until weaning and/or termination.

Dosing Solutions: A high dose suspension was obtained by mixing an appropriate amount of previously micronized test material with the vehicle (Silverson Labs. mixer emulsifier). Serial dilutions were made from it to obtain mid- and low dosing suspensions. All dosing suspensions were made daily, within 4 hours of dosing, and were constantly (magnetically) stirred during the dosing operation. Samples were retained on 9 occasions throughout the study (Premating wks 1, 3, 7, mating wk, and wk 3 of lactation for F0 rats; premating wks 5, 10, mating wk, and wk 2 of lactation for F1) for analysis of a.i. concentration. The analytical method used for determination of a.i. concentration in the dosing suspensions was not mentioned in the report. According to the results (Appendix 4 of report), mean achieved concentrations were acceptable (within 10% of target) except for two samples (F0; mating week; LD = 85% of target and MD = 88% of target). Coefficient of variations were acceptable (≤11%) except for one sample (F0; Wk 7; LD = 30%).

Feed and Water: The rats received feed [Rat breeder diet No. 3 SQC Expanded; Special Diets Services LTD ((SDS); Essex; U.K) and tap water ad libitum. Diet was analysed for nutritive constituents and pesticide contaminants (SDS) and water for bacterial contamination (Clayton Bostock Hill & Rigby). The results were satisfactory.

B. METHODS

I. STUDY DESIGN

Parental F0 (about 38-day old) and F1 (25-day old) rats were randomly assigned to the following groups:

Dose Groups+	mg/kg/day (oral gavage)	F0 Generation Males	F0 Generation Females	F1 Generation++ Males	F1 Generation++ Females
Control	0	28	28	28	28
Low dose	1.0	28	28	28	28
Mid dose	2.5	28	28	28	28
High dose+++	4.0	28	28	28	28

+: Siblings were not assigned to the same dose-group.
++: 24/sex/group used for breeding of F2 generation and 4/sex/group kept for replacements as needed.
+++: 5 mg/kg/day (F0 dosing days 1-16) reduced to 4 mg/kg/day (F0 dosing days 17-termination).
F0 and F1 rats were dosed through their respective premating (10 wks/F0s; 11 wks/F1s), mating, gestation, and lactation periods.

The dose range selection was based on 2 preliminary oral toxicity studies in rats of unspecified age, in which mild gastric mucosal irritation was the only adverse effect observed at 4 mg/kg/day (8-wk study at 0, 0.1, 0.5, 1 and 4 mg/kg/day) and severe unspecified toxicity was seen at ≥8 mg/kg/day (13-Wk study at 0, 1, 8, 16, and 24 mg/kg/day).

II. MATING, SELECTION OF F1s FOR BREEDING, AND TERMINATION OF STUDY: One male and 1 female of the same generation and dose group were housed together. Daily vaginal lavages were collected to determine the oestrus stage. Successful mating (Gestation day 0) occured at the first detection of sperm cells in a vaginal lavage and/or at detection of a copulatory plug in situ. If a sucessful mating did not occur after 7 days of cohabitation, then the male was removed and replaced 2 days later by another male of proven fertility. No further matings were attempted after 2 consecutive unsuccessful 7-day pairing periods. Pairing of siblings were avoided.

Pregnant F0 and F1 females were individually housed in cages with a solid bottom and bedding where they were kept through gestation and, with their litters, through lactation. F1 pups (28/sex/group) selected for production of the F2 generation were weaned at 25 days of age (Study termination for parental F0s and unselected F1 pups). After 6 weeks of direct dosing, the number of F1 pups was reduced to 24/sex/group, and the extra rats were discarded. All F2 pups remained with their mother until about lactation day 21 (Study termination for parental F1 and pups F2).

III. OBSERVATIONS

1. Parental (F0 and F1) Observations:

a. Clinical Observations: All animals were observed daily for clinical signs of toxicity. The onset, intensity, and duration of the signs were noted.

Mortality/Moribundity: All animals were observed twice daily for mortality/moribundity. Animals found moribund were sacrificed.

Body Weight: Individual body weight were recorded weekly for males (excluding the mating period) and during the premating period for females. Pregnant females were additionally weighed on gestation day 0, 7, 14, 16, and 20, and lactation day 1 (1 day after birth), 7, 14, 21, and at termination.

Feed Consumption: Individual feed consumption were measured weekly for males (excluding the mating period) and during the premating period for females. Feed consumption of pregnant females were additionally recorded during the periods from gestation days 0-7, 7-14, and 14-20 and lactation days 0-7 and 7-14.

Reproductive Parameters: Oestrus stages, precoital intervals, and duration of gestation/parturition were recorded and the following reproductive indexes (I) were calculated:

$$\text{Male fertility I (FI)} = \frac{\text{No. females pregnant}}{\text{No. males mated}} \times 100$$

$$\text{Female FI} = \frac{\text{No. females pregnant}}{\text{Total no. females mated}} \times 100$$

$$\text{Gestation I (GI)} = \frac{\text{No. live litters born}}{\text{No. pregnancies}} \times 100$$

2. LITTER (F1 and F2) OBSERVATIONS:

Litters were observed for the number of pups born alive or dead. Live pups were sexed and examined for gross abnormalities. Pups dead during the lactation period were examined externally and for the presence of milk in their stomach. Preweaning F1 and F2 pups of the same sex from each litter were weighed together on lactation days 1, 4, 7, 14, and 21 and also individually on lactation day 21.

The following indexes (I) were calculated:

$$\text{Birth I (BI)} = \frac{\text{Total No. pups born}}{\text{No. implantation scars}} \times 100$$

$$\text{Live BI (LBI)} = \frac{\text{No. live pups on lactation day 0}}{\text{No. pups born}} \times 100$$

$$\text{Viability I (VI)} = \frac{\text{No. live pups on lactation day 4}}{\text{No. pups born alive}} \times 100$$

$$\text{Lactation I (LI)} = \frac{\text{No. live pups on lactation day 21}}{\text{No. live on lactation day 4}} \times 100$$

$$\text{Overall Survival I (OSI)} = \frac{\text{No. live pups on lactation day 21}}{\text{No. born}} \times 100$$

IV. TERMINAL STUDIES

1. Parental F0 and F1 Animals: All surviving animals were sacrificed by carbon dioxide asphyxiation and were examined externally, then internally (in situ gross examination of tisues/organs within the cranial, thoracic, and abdominal cavities). Representative samples of all abnormally looking tissues were fixed in 10% neutral buffered formalin (NBF). The number of uterine implantation sites were recorded and reproductive tracts of non-parturient females were checked for signs of pregnancy. The following organs were fixed in NBF but were not examined microscopically:

X	Ovaries	X	Epididymides+
X	Uterus	X	Prostate+
X	Unusual lesions	X	Seminal vessicles+
X	Vagina/cervix	X	Testes+
X	Pituitary Gland	X	Adrenal Gland+

+ organs were also weighed.

2. F1 and F2 pups: All F1 weanlings not selected for breeding, all F2 weanlings, and all F1 pups dead/sacrificed in extremis before lactation day 14 were only examined for external alterations. All F1 pups dead/sacrificed in extremis on or after lactation day 14 were examined for external alterations followed by necropsy of tissues/organs within the thoracic and abdominal cavities.

V. DATA ANALYSIS

"Where required, tests were applied to determine the statistical significance of observed differences between treatment groups: Organ weight data were analyzed by analysis of variance and also by analysis of covariance, using the terminal weight as the single covariate, treatment groups being compared using the F-protected Least Significant Difference procedure".

C. RESULTS

I. PARENTAL (F0 AND F1) RESULTS

a. Mortality, Clinical Signs, and Necropsy Findings

In the F0 generation (Table 1), 4 HD rats (1 male and 3 females) were sacrificed in extremis, within the first 16 days of dosing. Their death was preceeded by clinical signs of breathing difficulty (labored breathing and/or wheezing; 4/4), piloerection (4/4), and abdominal distention (3/4) and their necropsy showed intestinal (3/4) or caecum (1/4) distension with gas. These consistent clinical signs and necropsy findings were considered to be treatment-related. Another HD male was also sacrificed in extremis during dosing week 12, but its death was not considered treatment-related since it only showed breathing difficulty and piloerection but not abdominal distention and necropsy findings were not related to the gastrointestinal (GI) tract (lung reddened). Other non treatment-related deaths (no adverse clinical signs preceeding death and no GI abnormalities observed at necropsy) included those of 1 MD male (sacrificed in extremis during dosing week 15), 1 MD female (found dead on lactation day 0), and 1 LD female (found dead on gestation day 23). Incidences of breathing difficulty, piloerection, and/or abdominal distention were also observed in some of the surviving MD and HD rats (Breathing difficulty: 1 MD and 3 HD; Piloerection: 2 MD and 2 HD; Abdominal distention : 1 HD). No mortality was observed in the control group. No adverse clinical signs similar to those described above were observed in the control and low-dose groups.

In the F1 generation (Table 2), 10 mortalities were observed within the first 3 weeks of direct dosing [2 LD males, 2 MD (one/sex), and 6 HD (3/sex). Necropsy findings of GI distention with gas was observed only in 1 MD female (ceacum distention) and 1 HD male (stomach distention) and these 2 deaths were considered to be treatment-related. One male MD was sacrificed in extremis during dosing week 18; this rat showed breathing difficulty and piloerection, but no GI abnormality at necropsy. One female HD was found dead during dosing week 18; its death was not preceeded by adverse clinical signs and no abnormal findings were seen at

necropsy. Incidences of breathing difficulty were also observed in 3 surviving high dose F1 rats (2 males and 1 female). No mortality was observed in the control group. No adverse clinical signs similar to those described above were observed in the control and low-dose groups.

Table 1. Fo Parental Mortality and Associated Clinical signs and Necropsy Findings

GROUP		MORTALITY No. (DW)	CLINICAL SIGNS (DW) Breathing Difficult*	Pilo-erection	Swollen Abdomen	NECROPSY FINDINGS Lung Reddened	GI Organ Distented With Gas	Other
CTRL	♂	0						
	♀	0						
LD	♂	0						
	♀	1a (14)c	-	-	-	Yes	-	Trachea froth filled, 13 dead full term fetuses
MD	♂	1b (15)	(14-15)**	-	-	-	-	Enlarged liver/spleen/kidney's pelvis
	♀	1a (15)d	-	-	-	-	-	Cause undetermined
HD+	♂	1b (3)	(3)	(3)	(3)	-	Intestines	
	♂	1b (12)	(9-12)	(9-12)	-	Yes	-	
	♀	1b (3)	(2-3)	(2-3)	(3)	-	Intestines	
	♀	1b (3)	(2-3)	(2-3)	(3)	-	Intestines	
	♀	1b (3)	(3)	(3)	-	-	Caecum(c)	

Data excerpted from Appendix 5 of report.
DW: Dosing week event was observed
+: HD = 5 mg/kg/day (dosing day 1-16) and 4 mg/kg/day (dosing day 17-end of study).
*: Labored breathing and/or wheezing; **: Breathing shallow/rapid.
a: Found Dead; b: Sacrificed in extremis.
c: Gestation day 23; d: Lactation day 0
- Not observed.

Table 2. F1 Parental Mortality and Associated Clinical signs and Necropsy Findings

GROUP		MORTALITY No. (DW)		CLINICAL SIGNS (DW) Breathing Difficult*	Pilo-erection	Swollen Abdomen	NECROPSY FINDINGS Lung Reddened	GI Organ Distented With Gas	Other
CTRL	♂	0							
	♀	0							
LD	♂	1a	(2)	-	-	-	-	-	Slightly cannibalized
	♂	1a	(2)	-	-	-	-	-	Slightly cannibalized
	♀	1b	(18)	-	(18)	-	-	-	Mammary tissue prominent.
MD	♂	1a	(1)	-	-	-	-	-	No abnormality detected
	♂	1b	(18)	(18)	(18)	-	-	-	Liver enlarged/friable
	♀	1a	(2)	-	-	-	Yes	Caecum	Lungs dark and spongy
HD+	♂	1a	(1)	-	-	-	-	Stomach	
	♂	1a	(1)	-	-	-	-	-	Lungs contained froth
	♂	1a	(3)	-	-	-	-	-	Lungs dark/spongy; froth in trachea
	♂	1a	(17)	-	-	-	-	-	No abnormality detected
	♀	1a	(2)	-	-	-	-	-	Red staining nose/limbs
	♀	1a	(1)	-	-	-	-	-	Brown staining around nose

Data excerpted from Appendix 6 of report. +: HD = 4 mg/kg/day.
DW: Dosing week event was observed
+: HD = 4 mg/kg/day.
*: Labored breathing.
a: Found Dead; b: Sacrificed in extremis.
- Not observed

12

b. Body weight and Body Weight Gain: Body weight and body weight gains of both F0 (Table 3) and F1 (Table 4) parental animals were comparable between groups, during the entire course of the study.

Table 3. Representative Group Mean (± SD) Body Weight (BW) and BW gain of F0 Rats.

Group	Control	Low dose	Mid dose	High dose
		MALES		
BW (g)				
Dosing wk 0	210 ± 26	215 ± 25	214 ± 22	212 ± 19
Dosing wk 10	521 ± 51	536 ± 54	536 ± 74	531 ± 47
Dosing wk 16	591 ± 60	612 ± 68	604 ± 87	606 ± 47
BW gain (g)				
Wk 0 - 16	381 ± 49	397 ± 54	392 ± 71	395 ± 47
		FEMALES PRE-MATING PERIOD		
BW (g)				
Dosing wk 0	171 ± 16	167 ± 20	164 ± 21	161 ± 19
Dosing wk 10	324 ± 41	311 ± 37	311 ± 33	304 ± 33
BW gain (g)				
Wk 0 - 10	153 ± 32	144 ± 25	147 ± 22	149 ± 23
		FEMALES GESTATION (G) AND LACTATION (L) PERIODS		
BW (g)				
G Day 0	329 ± 44	310 ± 35	320 ± 33	311 ± 39
G Day 20	483 ± 52	465 ± 46	470 ± 41	459 ± 44
L Day 1	362 ± 45	351 ± 45	346 ± 33	340 ± 41
L Day 14	398 ± 37	389 ± 37	389 ± 31	390 ± 31
L Day 21	389 ± 33	377 ± 34	376 ± 27	375 ± 31
BW gain (g)				
G Days 1-20	152 ± 24	157 ± 18	151 ± 26	149 ± 22
L Days 1-14	37 ± 21	42 ± 18	43 ± 20	46 ± 26

Data excerpted from tables 3-5 of report.
Nos./sex/group: 25-28 males throughout study or females during premating period; 17-27 females during gestation and/or lactation periods.

Table 4. Representative Group Mean (± SD) Body Weight (BW) and BW gain of F1 Rats.

Group	Control	Low dose	Mid dose	High dose
		MALES		
BW (g)				
Dosing wk 0	107 ± 17	105 ± 15	103 ± 20	103 ± 15
Dosing wk 11	536 ± 52	529 ± 43	522 ± 58	505 ± 54
Dosing wk 18	618 ± 74	626 ± 55	618 ± 70	602 ± 61
BW gain (g)				
Wk 0 - 18	509 ± 72	521 ± 55	514 ± 66	497 ± 59
		FEMALES PRE-MATING PERIOD		
BW (g)				
Dosing wk 0	100 ± 14	94 ± 16	96 ± 14	95 ± 17
Dosing wk 11	300 ± 29	296 ± 33	294 ± 35	295 ± 27
BW gain (g)				
Wk 0 - 11	201 ± 22	202 ± 30	195 ± 27	198 ± 25
		FEMALES GESTATION (G) AND LACTATION (L) PERIODS		
BW (g)				
G Day 0	303 ± 30	295 ± 30	283 ± 26	294 ± 27
G Day 20	455 ± 39	441 ± 43	437 ± 36	447 ± 44
L Day 1	343 ± 30	336 ± 38	333 ± 36	346 ± 37
L Day 14	386 ± 31	380 ± 37	373 ± 28	379 ± 34
L Day 21	365 ± 34	361 ± 37	353 ± 22	346 ± 40
BW gain (g)				
G Days 1-20	152 ± 24	146 ± 24	154 ± 23	153 ± 23
L Days 1-14	43 ± 18	45 ± 20	41 ± 25	33 ± 19

Data excerpted from tables 9-11 of report.
Nos./sex/group: 21-24 males throughout study or females during premating period; 18-23 females during gestation and/or lactation periods.

c. Feed Consumption (Table 5) : Feed consumption of both F0 and F1 parental rats were comparable between groups, during the entire course of the study.

Table 5. Representative Group Mean Feed Consumption (g/rat/week) of F0 AND F1 Rats.

Group	F0 PARENTAL RATS				F1 PARENTAL RATS			
	Control	LD	MD	HD	Control	LD	MD	HD
				MALES				
Dosing wk 0	179	183	185	184				
Dosing wk 1	211	215	211	210	141	142	143	143
Dosing wk 10	206	210	208	203	239	240	240	229
Dosing wk 15	273	280	276	280	258	264	250	259
Dosing wk 16	207	207	206	208	246	253	241	245
				FEMALES				
Dosing wk 0	150	145	141	144				
Dosing wk 1	157	151	146	144	129	128	130	131
Dosing wk 7	172	168	166	154	158	162	162	163
Dosing wk 10	147	143	139	144	153	161	147	146
G Days 0-7	196	187	185	184	180	172	175	183
G Days 4-14	217	204	208	220	208	198	197	209
G Days 14-20	193	184	188	189	189	181	197	199
L Days 0-7	274	274	271	262	289	279	281	284
L Days 4-14	508	492	485	495	501	473	514	471

Data excerpted from tables 6-8 (F0) and 12-14 (F1) of report. SDs of the means were not reported.
G day: Gestation day; L day: Lactation day.
Refer to tables 1 and 2 for Nos. of rats/sex/group

d. Reproductive Parameters (Table 6): All reproductive parameters examined, including precoital interval, oestrus stage, male/female fertility indexes, duration of gestation/gestation index, the numbers of dams with a live litter, implants/pregnancy, and pups born (dead or alive)/per dam were comparable between the respective F0 and F1 groups.

Table 6. Reproductive Parameters in F0 and F1 Parental Groups.

Dose Group		F0 GENERATION CTRL	LD	MD	HD	F1 GENERATION CTRL	LD	MD	HD
Observation									
Median Precoital Interval (days)		2.2	2.0	3.0	3.0	3.0	3.0	3.0	3.0
No. Passing 1 Oestrus		1	1	0	0	4	1	3	1
No. Passing 2 Oestruses		1	0	0	0	0	1	1	0
No. Males Paired		28	28	28	25	24	24	24	24
No. Males Fertile		24	28	20	23	23	21	19	23
Male FI as %		86	100	71	92	96	88	79	96
No. Females Paired		28	28	28	25	24	24	24	24
No. Pregnant		26	28	21	25	24	22	20	23
Female FI as %		93	100	75	100	100	92	83	96
Mean Duration of Gestation (days)		21.9	21.8	21.7	22.0	21.8	21.9	21.7	21.9
No. Females With Live Litter		26	27	21	25	24	22	20	23
GI as %		100	96	100	100	100	100	100	100
No. Implants /Pregnancy	**Mean**	16.4	16.9	16.1	15.5	15.8	15.9	15.0	15.0
	SD	2.8	2.2	2.6	3.8	2.5	1.6	3.3	2.2
No. Pups Born Dead or alive/Dam	**Mean**	15.2	15.4	14.5	14.3	14.4	14.5	14.2	13.9
	SD	3.2	2.7	2.5	3.7	3.1	1.9	2.8	2.0

Data excerpted from tables 15-18 of report.
SDs of groups means were not reported except when indicated above.
FI = Fertility index; GI = Gestation index.

e. Terminal Studies

1. Organ Weight: Group mean organ (epididymides, prostate, seminal vesicles, testes, and adrenal gland) weight (absolute or adjusted for body weight) values were comparable between the respective F0 and F1 rats groups.

2. Necropsy: Necropsy results were described earlier (See C.I.a. Section above).

3. Histopathology: Histopathology of the reproductive organs was not done.

II. LITTER (F1 AND F2) RESULTS

a. Observations (Tables 7-8): All F1 and F2 litter observations, including the number of live pups/litter on lactation days 0, 1, 4, 7, 14, or 21, the birth, live birth, viability, lactation, and overall survival indexes, and the groups/litter mean weights were comparable between the respective F1 or F2 litters groups.

b. Terminal Studies

No summary table/individual data were provided. The report stated that no external abnormalities were observed in any of the F1 pups not selected for breeding or the F2 pups and these pups were therefore discarded.

The following single incidences of abnormalities among pups were also listed in the report:

- "Umbilical hernia: 1 control F1,
- Apparent (in error, not checked at necropsy) left anopthalmia: 1 control F1,
- Right eye protruding: 1 MD F1,
- Absent tail: 1 HD F1,
- Apparent (not confirmed at necropsy) Left eye prior to weaning: 1 MD F2, and
- Left anopthalmia: 1 MD F2".

Table 7. Number of Live Pups/litter on Lactation Days 0, 4, 14, and 21 and Respective Survival Indexes of F1 and F2 Litter Groups.

		F1 LITTERS				F2 LITTERS			
Dose Group		**CTRL**	**LD**	**MD**	**HD**	**CTRL**	**LD**	**MD**	**HD**
Observation									
No. Live Pups/Litter									
LD 0	**Mean**	15.1	15.2	14.4	14.1	14.4	14.4	14.1	13.8
	SD	3.1	2.6	2.5	3.7	3.1	1.9	2.9	2.0
LD 4	**Mean**	14.2	14.1	13.1	12.8	14.0	13.7	13.8	13.1
	SD	2.9	2.6	3.2	3.9	3.1	2.7	2.7	2.4
LD 14	**Mean**	13.3	13.2	12.0	11.7	13.9	12.9	13.8	12.8
	SD	3.0	3.1	3.7	3.7	3.0	3.4	2.7	2.1
LD 21	**Mean**	13.3	13.2	12.0	11.7	13.9	12.9	13.8	12.7
	SD	2.9	3.2	3.7	3.7	3.0	3.4	2.7	2.1
Mean Litter BI as %		92	92	91	92	89	90	94	93
Mean Litter LBI as %		99	99	99	98	100	98	99	99
Mean Litter VI as %		94	90	84	92	94	87	91	95
Mean Litter LI as %		95	93	87	93	100	90	95	97
Mean Litter OSI as %		89	82	75	85	93	79	93	92

Data excerpted from tables 17-20 of report.
SDs of groups means were not reported except when indicated above.
LD = Lactation Day; BI = Birth index; LBI = Live birth index; VI = Viability index; LI = Lactation index; OSI = Overall survival index.

Table 8. Group Mean Pup and Litter Weight (g) on Lactation Days (LD)1, 4, 14, and 21.

Dose Group		F1 LITTERS CTRL	F1 LITTERS LD	F1 LITTERS MD	F1 LITTERS HD	F2 LITTERS CTRL	F2 LITTERS LD	F2 LITTERS MD	F2 LITTERS HD
				GROUP WEIGHT					
Males LD 1	Mean	6.6	6.5	6.6	6.8	7.0	6.8	6.9	6.7
	SD	0.5	0.7	0.7	0.8	0.8	0.7	0.8	0.6
Females LD 1	Mean	6.1	6.1	6.2	6.4	6.6	6.3	6.4	6.3
	SD	0.6	0.7	0.7	0.7	0.8	0.7	0.5	0.6
Males LD 4	Mean	9.5	9.1	9.0	9.8	10.4	9.3	10.4	9.7
	SD	1.3	1.4	1.8	1.8	1.7	1.8	1.4	0.9
Females LD 4	Mean	8.8	8.5	8.5	9.2	9.8	8.8	9.6	9.3
	SD	1.4	1.4	1.7	1.4	1.8	1.6	1.2	1.0
Males LD 14	Mean	28.4	27.1	27.7	28.4	28.3	26.5	28.0	27.4
	SD	4.0	3.3	3.0	4.7	4.4	3.2	3.5	3.1
Females LD 14	Mean	27.2	25.5	26.2	27.3	26.9	26.2	26.4	26.5
	SD	4.3	3.0	3.0	4.6	4.6	4.2	3.7	3.4
Males LD 21	Mean	45.3	43.2	44.9	46.4	45.0	41.8	44.4	43.0
	SD	7.8	6.4	5.2	8.8	9.3	6.3	6.9	5.6
Females LD 21	Mean	42.9	41.0	42.2	44.1	42.3	40.4	41.7	41.7
	SD	7.3	6.1	5.0	8.3	9.1	6.2	7.1	5.6
				LITTER WEIGHT					
LD 1	Mean	93	93	90	86	96	95	93	87
	SD	19	14	17	25	20	15	17	16
LD 4	Mean	128	125	116	119	139	126	137	126
	SD	27	28	42	36	30	35	25	28
LD 14	Mean	358	342	326	316	375	333	368	343
	SD	70	68	104	79	59	84	55	51
LD 21	Mean	568	530	517	507	586	522	581	535
	SD	104	104	158	117	87	121	83	80

Data excerpted from tables 21-24 of report.

D. DISCUSSION

I. INVESTIGATORS' DISCUSSION/CONCLUSIONS

The following discussion/conclusions are directly quoted from the report:

"Treatment with 5.0 mg MTC.Kg^{-1}.day^{-1} produced severe toxicity: 4 F0 animals were killed due to poor condition during the third week of treatment. Continued administration of 5.0 mg MTC.Kg^{-1}.day^{-1} was considered to be inappropriate and the high dose level was lowered to 4.0 mg MTC.Kg^{-1}.day^{-1}".

"Nine F1 animals, all in MTC-treated groups, died in the first 2 weeks of direct treatment. Of these, 4 (one at 2.5 mg, 3 at 4.0 mg MTC.Kg^{-1}.day^{-1}) had shown lung and/or gastrointestinal disturbances at necropsy similar to those seen in F0 decedent animals that had received 5.0 mg MTC.Kg^{-1}.day^{-1}. It was therefore considered that these deaths were probably associated with treatment. The cause of death of the other 5 (2 at 1.0 mg, one at 2.5 mg, 2 at 4.0 mg MTC.Kg^{-1}.day^{-1}) of these 9 animals was not determined, but it was considered that death was associated either with the dosing technique or the test material. Since those deaths that were clearly caused by the test material were associated with gaseous distension of the gastro-intestinal tract, it was considered that the deaths of those 5 animals where cause of death was not established were probably due to dosing accidents".

"Under the conditions of this study, the no effect level for reproductive function, including pup survival and development, was 4.0 mg MTC.Kg^{-1}.day^{-1} (the highest level tested). The no effect level for parental effects was 1.0 mg MTC.Kg^{-1}.day^{-1}".

II. REVIEWER'S DISCUSSION

The reviewer agrees with the investigators'conclusions regarding the establishment of a parental systemic NOEL at 1.0 mg/kg/day (LD) and a reproductive NOEL at 4.0 mg/kg/day (HD). The parental systemic LNOEL was 2.5 mg/kg/day (MD), based on one F1 (female) mortality associated with necropsy findings of caecum distention with gas.

The reviewer also agrees with the investigator's conclusions regarding the consideration of "gaseous distention of the gastrointestinal tract" as a criteria for treatment-related death since 1) "those deaths that were clearly caused by the test material were associated with gaseous distension of the gastrointestinal tract", 2) this necropsy finding was observed in rats which were sacrificed in extremis, thus precluding a false

positive GI gaseous distention caused by bacteria, and 3) MTC is known to cause severe irritation of the gastrointestinal system [Preliminary 8-wk oral study (this report); Developmental studies in rabbits and rats (MRID Nos. 411719/01-02).

It is difficult to obtain a good dose range for a chronic study with MTC since this pesticide is known to have a low margin of safety [In the present study, the dose causing excessive mortality (5 mg/kg/day) was low and was only 5 times the NOEL. In a developmental study in rabbits (MRID No. 411719-02) the dose 7 mg/kg/day caused excessive mortality and was only twice the NOEL]. Consequently the dose range used in this study is considered to be adequate; although the initial high dose (5 mg/kg/day) used was too high (5 mortalities within 16 days of dosing), it was adequately reduced to yield an acceptable number of pregnant dams per dose group.

The conduct of the study was generally adequate except for several misdosings [F0 generation: 2 MD given control diet and 2 Control given MD diet for 4 consecutive days (dosing wk 3); 1 LD dosed twice the same day (lactation day 4); 1 LD (dosing wk 15) and 1 MD (dosing wk 17) given less than their dose because of incorrect intubation. F1 generation: 1 HD given control diet for 4 consecutive days (at 7 wk of age)].

Two deviations from acceptance criteria were noted, including 1) The age of F0 rats (about 38 days) at study initiation (They were younger than the age suggested (8 wks) in guideline 83-4), and 2) The absence of reproductive organs histopathology (guideline 83-4 suggests reproductive organs histopathology for at least the control and high dose groups). The first deviation is minor. Because no adverse effect on non-histological reproductive parameters were observed, the second deviation was considered not detrimental to the integrity of the study. Nevertheless, histological data can only add to the scientific value of this study.

Two deficiencies in the reporting of the study were also noted, including 1) No mention of the analytical method used to analyse the a.i. concentration in the dosing suspensions, and 2) No summary table/individual data on litter terminal studies were included in the report. The investigators did provide a list of incidence of abnormalities among pups (See Section C.II.b.).

Because of the above deviations/deficiencies, this study is considered to have minimally satisfied the toxicological data requirements for a 2-generation reproductive toxicity study in rats (83-4) and is core classified Minimum.