PC 100301

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

006385

SEP 8 1987

MEMORANDUM:

SUBJECT: I.D. 100-510: Methidathion: Miscellaneous toxicity data required for the Registration Standard.
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Tox. Chem. No. 378B

TO: Dennis Edwards (PM 12)
Registration Division (TS-767C)

FROM: Marion P. Copley, D.V.M., D.A.B.T. Marion P. Copley 8/27/87
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Judith W. Hauswirth, Ph.D., Section Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C) Judith W. Hauswirth 8/27/87

Ciba-Geigy has submitted several toxicity studies for review in order to satisfy requirements for registration of the insecticide, Methidathion. These studies include:

1) 2-year chronic/oncogenicity mouse study,

2) two reproduction studies,

3) rat teratology study, and

4) rabbit teratology study.

WJB 9/4/87

The chronic/onco mouse study is considered core-minimum and satisfies the guideline requirement (83-5) for a rodent oncogenicity and chronic feeding study. This study demonstrated that Methidathion may be an oncogen producing liver tumors in male mice. This issue will be presented to the Toxicology Branch Peer Review Committee in late 1987. Although the rat reproduction study # 450-1713 is supplementary, study # 450-2125 is core-minimum and satisfies the guideline requirement (83-4) for a 2-generation reproduction study. The rat and rabbit teratology studies are both core-minimum and satisfy the requirements for 2 teratology studies (83-3). See table 1 for a summary of the study results.

The registration standard (FRSTR) for Methidathion is due to be completed in May 1988. At that time the entire data base for Methidathion will be reevaluated and any remaining data gaps will be determined.

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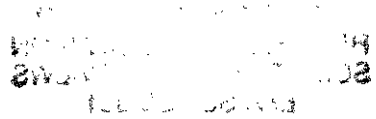

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TABLE 1 CURRENTLY SUBMITTED DATA WITH TECHNICAL METHIDATHION

Study	Results
Chronic/onco (2 year)-mouse 382-087 core-minimum	NOEL = 10 ppm (1.6 mg/kg/day) LEL = 50 ppm (7.5 mg/kg/day) based primarily on non-neoplastic liver alterations (males), Che (RBC) inhibition (females) Oncogenicity = males only; increased hepatocellular adenomas and carcinomas, when considered separately at the high dose, and at the mid and high dose when they are combined.
Reproduction 2-generation-rat 450-2125 core-minimum	NOEL (parental) = 5 ppm LEL (parental) = 25 ppm based primarily on tremors and decreased food consumption during lactation. NOEL (reproductive) = 5 ppm LEL (reproductive) = 25 ppm based primarily on unthriftyness in the pups while nursing.
Reproduction 1-generation-rat 450-1713 core-supplementary	NOEL (parental) = 5 ppm LEL (parental) = 50 ppm based primarily on tremors and decreased food consumption during lactation. NOEL (reproductive) = 5 ppm LEL (reproductive) = 50 ppm based primarily on decreased pup survival and tremors in the pups.
Teratology -rabbit 86131 core-minimum	NOEL (maternal) = 6 mg/kg/day LEL (maternal) = 12 mg/kg/day based primarily on clinical signs of cholinesterase inhibition. NOEL (developmental) $\geq$ 12 mg/kg/day (high dose). LEL (developmental) not achieved in this study.
Teratology -rat 86172 core-minimum	NOEL (maternal) = 1.00 mg/kg/day LEL (maternal) = 2.25 mg/kg/day based primarily on clinical signs of cholinesterase inhibition. NOEL (developmental) $\geq$ 2.25 mg/kg/day (high dose). LEL (developmental) not achieved in this study.

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WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 100301, Chronic Toxicity Study in Dogs with Methidathion (GS13005)

Tox. Chem. No.: 378B
Project No.: 1-1839
Record No. : S399918

FROM: Melba S. Morrow, D.V.M.
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Dennis Edwards, PM 12
Registration Division (H7505C)

THRU: Joycelyn E. Stewart, Ph.D.
Section Head, Review Section II
Toxicology Branch I
Health Effects Division (H7509C)

9/24/91

9/24/91

CONCLUSIONS:
Based on the results of this chronic study in beagle dogs, the NOEL is 4 ppm (0.15 mg/kg) and the LOEL is 40 ppm in both sexes based on the elevation of hepatic enzymes, gross hepatic lesions and the microscopic presence of bile plugs, distended bile canaliculi and chronic hepatitis. In addition, in males, RBC cholinesterase levels were also depressed (27 -30%) at 40 ppm.

At 140 ppm, findings similar to those observed at 40 ppm were present in both sexes with regard to clinical chemistry, gross and microscopic pathology. In both sexes, RBC and brain cholinesterase levels were also depressed when compared to controls at this dose.

The study is classified as core minimum because on several occasions, the concentration of the test substance in the diet fell outside of the acceptable range.

A copy of the DER is attached for your reference.

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Reviewed by: Melba S. Morrow, D.V.M. 008619
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. 9/21/91
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity-Dog

GUIDELINE #: 83-1

TOX. CHEM. #: 378B

MRID #: 419450-01

TEST MATERIAL: GS13005, 96% Active Ingredients

SYNONYMS: Methidathion

STUDY NUMBERS: F00028

SPONSOR: Ciba Geigy
Greensboro, North Carolina

TESTING FACILITY: Ciba Geigy
Farmington, Connecticut

TITLE OF REPORT: One Year Dietary Toxicity in Beagle Dogs (EHC Laboratory Study Number F-00028)

AUTHORS: Jane C.F. Chang and James A. Walberg

REPORT ISSUED: June 24, 1991

CONCLUSIONS: Based on the results the NOEL is 4 ppm (0.15 mg/kg) and the LEL is 40 ppm (1.33-1.39 mg/kg) based on elevation of liver enzymes, gross discoloration of the liver and the microscopic presence of bile plugs, distended bile canaliculi and chronic hepatitis. In males receiving the test material at 40 ppm, erythrocyte cholinesterase levels were also depressed (27 - 30 %) when compared to controls.

CLASSIFICATION: Minimum
The information presented for this chronic nonrodent study satisfies the criteria set forth in Subdivision F, Series 83-1.

MATERIALS: GS13005, a waxy, pale yellow material, with a purity of 96% was the test material. The lot number was F1-890331. The compound is an organophosphate insecticide.

The test animals were Beagles, purchased from Marshall Farms in North Rose, N.Y. The animals were approximately 5 months of age at the start of the study. Males weighed 6.4 to 8.8 kg and

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females weighed 5.5 to 8.5 kg.

METHODS: Animals were identified, housed individually and maintained in an environment with an average temperature of 19 to 24° C and a relative humidity of 40 to 60%. Animals underwent an acclimation period of 28 days, during which time, they were subjected to a health assessment which included physical, ophthalmological and fecal examinations. Hematology, urinalysis, and clinical chemistry parameters were also evaluated prior to the adminstration of the test compound.

Four dogs per sex were allocated to the following treatment groups and received the daily doses of the test material in their feed:

Dose (ppm)	Dose (mg/kg)
0.5	0.02
2.0	0.07
4.0	0.15
40.0	1.33(M), 1.39(F)
140.0	4.51(M), 4.90(F)

The doses for this chronic study were selected based on the results of a 90 day study in which levels of 0, 0.5, 4.0, 45 and 140 ppm were fed. At 45 and 140 ppm, elevations in liver enzymes were reported and at necropsy, corresponding gross lesions were present in the livers of animals from these two dose groups. Microscopically, cholestasis was diagnosed. Erythrocyte cholinesterase activity was significantly inhibited at the highest dose tested (88%); however, there was no observed effect on the brain and serum cholinesterase levels at the highest dose tested.

Animals were observed twice daily for general appearance, behavior, signs of toxicity and mortality. Physical examinations were conducted every 3 months (3,6,9 and 12) and consisted of measurements of temperature, pulse and respiration. Weekly general examinations were conducted which involved palpation of each animal to determine the presence of tissue masses.

Hematology, clinical chemistry and urinalysis were conducted at 3 and 6 months and at termination. Animals were fasted for 16 hours prior to the collection of blood.

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The following (x) hematology, clinical chemistry and urinalysis parameters were evaluated:

x Hematocrit (HCT)
x Hemaglobin (HGB)
x Leukocyte count (WBC)
x Erythrocyte count (RBC)
x Platelet count
x Leukocyte differential
x Mean corpuscular hemaglobin
x Mean corpuscular hemaglobin concentration
x Mean corpuscular volume
x Reticulocytes

Blood clotting measurements:
x Thromboplastin time
Clotting time
x Prothrombin time

Electrolytes:
x Calcium
x Chlorine
Magnesium
x Phosphorus
x Potassium
x Sodium

Enzymes:
x Creatinine phophokinase
x Alkaline phosphatase
x Lactic dehydrogenase
x SGPT
x SGOT
x Gamma glutamyl trans.
x sorbitol dehydrogenase
x Cholinesterase

Other Serum Chemistry Values:
x Albumin
x Blood creatinine
x BUN
x Cholesterol
x Globulin
x Glucose
x Total Bilirubin
x Total protein
x Triglycerides
Serum protein electrophoresis

Urinalysis
x Volume
x Color/appearance
x spec. gravity
x pH
x protein
x glucose
x ketones
x bilirubin
x urobilinogen
x occult blood

Food consumption was measured twice weekly during the first 13 weeks and twice per month during the remainder of the study. Body weights were determined weekly and at the termination of the study.

At the end of 12 months, the dogs were anesthetized with sodium pentobarbitol and euthanized by exsanguination. A full gross necropsy was performed on all animals. Brain, heart, spleen, pituitary, liver, kidneys, adrenals, thyroid (with parathyroid) and testes /ovaries were weighed. Tissue from these same organs along with tissues from the lung, spleen and skin were preserved in 10% neutral buffered formalin or 25% buffered glutaraldehyde. Tissues were sectioned, stained with hematoxylin and eosin and and submitted for histopathological examination. The severity of the lesions was graded by the pathologist on a scale of 1 (slight changes) to 4 (marked or dominant changes).

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The following CHECKED (x) tissues were collected for histological examination. Weighed organs are designated by (xx)

Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	x Aorta	xx Brain
x Salivary glands	xx Heart	x Periph. nerves
x Esophagus	x Bone marrow	x Spinal cord
x Stomach	x Lymph nodes	
x Duodenum	xx Spleen	
x Jejunum	xx Thymus	Glandular
x Ileum		xx Parathyroids
x Cecum		xx Adrenals
x Colon	Urogenital	xx Thyroid
x Rectum	xx Kidneys	xx Pituitary
	x Urinary bladder	x Mammary(f)
xx Liver	xx Testes	
x Gall bladder	x Epididymides	Other
x Pancreas	x Prostate	x Bone
	x Seminal vesicle	x Skin
Respiratory	xx Ovaries	x Skel. muscle
x Trachea	x Uterus	x All gross lesions
x Lung	x Vagina	x Eyes
x Nasal passages		
Pharynx		
Larynx		

STABILITY, HOMOGENEITY and CONCENTRATION:

The diet was prepared by mixing the technical GS130005 with Purina Certified Canine Diet. The test material was melted at approximately 50° C and was mixed with the corn oil and added to the Purina diet. Test diets were blended once every two weeks. The prepared diets were stored at 4° C until they were given to the animals. Food was provided to each animal for approximately 4 hours each day.

The stability of the test substance in the diet was determined prior to the initiation of the study. Concentration and homogeneity were measured approximately once a month.

STATISTICAL ANALYSIS:

One -way ANOVA followed by two-way Dunnett's "T" was performed on body weights, body weight gains, food consumption, hematology, clinical chemistry, organ weights, urine pH, urine volume and specific gravity. ($p \leq 0.05$, $p \leq 0.01$).

QUALITY ASSURANCE:

A statement of quality assurance has been included in the submission. A statement of compliance with GLPs has also been included.

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RESULTS:

Body Weight, Body Weight Gains and Food Consumption:

No treatment related effects on body weights, body weight gains were reported. Food consumption was lower for males in the high dose group; however feed efficiency was not adversely affected when compared to controls.

Clinical Signs:

Salivation, diarrhea and dacryorrhea (excessive production of tears), were the most frequently observed clinical signs. However, none of the signs showed a dose response relationship. (See Table I).

Hematology, Clinical Chemistry and Urinalysis:

In males receiving GS 13005 at levels of 40 and 140 ppm a decrease in the number of neutrophils with a corresponding increase in the number of lymphocytes was observed at the three month sampling period. In addition these findings did not occur at the 6 and 12 month sampling periods.

At 40 and 140 ppm, liver enzymes were elevated in both males and females to biologically significant levels for alkaline phosphatase, SGPT, SGOT, and sorbitol dehydrogenase. Bilirubin was also slightly increased. In females in these two groups, increases in gamma glutamyl transferase were reported along with decreases in total protein and serum albumin. The elevations in hepatic enzymes and serum bilirubin are indicative of hepatocellular damage with accompanying cholestasis. The decreases in total protein and serum albumin in females are also indicative of liver disease. (See Table II for hepatic enzyme levels in controls and in dogs receiving GS13005 at levels $\geq$ 40 ppm).

No alterations in plasma cholinesterase activity were reported throughout the study. Red blood cell cholinesterase was depressed in males at 40 ppm (26.6 to 30%) and 140 ppm (77.3 to 87%) and at 140 ppm (76 to 83%) in females. Brain cholinesterase was depressed in both sexes at 140 ppm (16.6 to 26.7%). Statistical significance was reached for both blood and brain cholinesterase activity at 140 ppm. (See Table III for comparison of control and treated animals).
The decreases in cholinesterase are related to the administration of the test compound.

No treatment related effects were observed in the urinalysis for either sex.

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Gross Pathology, Histopathology:

Grossly, the most significant observation was the dark red discoloration of the livers in males and females in the two highest dose groups. Two of four females and one of four males at 40 ppm and three of four females and two of four males at 140 ppm were affected.

Incidental increases in absolute and relative spleen weights and relative increases in kidney weights were reported in males receiving 4 ppm. These findings are not considered treatment related.

A papilloma was observed in one male dog which received the test compound at 4 ppm. The occurence of this neoplasm is not related to the administration of the test compound. These benign tumors are commonly observed in young dogs, are associated with a virus and undergo spontaneous regression.

On histological examination, cholestasis, characterized by the presence of bile plugs and distended bile canaliculi was observed in the centrilobular zone of the livers in animals receiving GS13005 at levels of 40 ppm and greater. Chronic hepatitis was also present in one male and three females in the 40 ppm group and one female in the 140 ppm group.

All micro and macroscopic findings can be correlated to the clinical chemistry values observed in animals in the two high dose groups.

Analysis for Concentration and Homogeneity:

A total of fourteen samples were analyzed for each dose level. Concentrations in the high dose group fell outside of the acceptable limits on four occasions (11 to 19% lower than the target concentration). The low dose was outside of the acceptable range on two occasions (27 - 33%) and the 2.0 ppm level was outside of the acceptable range on one occasion (22%).

With regard to homogeneity, only one sample at the 4 ppm dose level was outside of the acceptable range. Overall, the concentration and homogeneity was acceptable for most of the study.

DISCUSSION:

The clinical chemistry parameters would lead to a diagnosis of hepatocellular damage with accompanying bile cholestasis. Acute biliary obstruction is one of the common causes of elevations in SGPT levels of greater than three times the normal level. In cases of biliary obstruction leading to intrahepatic cholestasis, elevated SAP and bilirubin levels can also be expected.

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Increases in SGOT, while not specific for hepatic injury when considered alone, may be indicative of hepatic disease when there is an accompanying elevation in SGPT levels.

Observed increases in GGT, decreases in albumin and total protein in females receiving the test material at levels greater than and equal to 40 ppm also suggests that there is hepatic damage.

The depressions in the red blood cell (76 to 87% depression at the highest dose tested) and brain (16.6 to 26.7% at the highest dose tested) cholinesterase levels can also be associated with the administration of the compound and may be related to the alteration in hepatic function.

The results of the gross and histopathology examinations confirm that which was diagnosed based on clinical chemistry parameters.

With regard to hematology values in males receiving 40 and 140 ppm, the reported decrease in neutrophils with a corresponding increase in lymphocytes is not biologically significant. These values (4014 and 4086 absolute lymphocytes and 5146 and 5668 neutrophils, for 40 and 140 ppm, respectively) are within the normal canine reference range (Small Animal Clinical Diagnosis by Laboratory Methods, Willard, et.al.).

Based on the results from this study the NOEL for both male and female beagles is 4.0 ppm. The LOEL for both sexes is 40 ppm based on the reported elevation in liver enzymes, the gross finding of discoloration of the liver and the microscopic presence of bile plugs, distended bile canaliculi and chronic hepatitis.

The study is classified as core minimum based on the fact that the concentration and homogeneity were outside of the acceptable range on several ocassions. As presented, the study satisfies the requirements set forth in Subdivision F Guidelines, 83-1 for chronic testing.

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TABLE I
FREQUENCY OF CLINICAL OBSERVATIONS

MALES ppm	0	0.5	2.0	4.0	40.0	140.0
OBS.						
Dacryorrhea	0	1/1*	4/3	3/1	6/2	0
Salivation[a]	2/1	2/1	5/2	1/1	6/3	7/3
Diarrhea/ Loose stool	3/1	2/2	2/1	8/2	0	2/1

FEMALES ppm	0	0.5	2.0	4.0	40.0	140.0
OBS.						
Dacryorrhea	0	1/1	2/1	2/1	4/2	0
Salivation[a]	0	0	7/3	4/2	1/1	10/4
Diarrhea/ Loose stool	1/1	1/1	1/1	1/1	0	1/1

* = the numerator represents the number of times the observation was made; the denominator represents the number of animals in which the observation was made.

a = salivation includes observations of wet face and forepaws.

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TABLE II
HEPATIC ENZYME LEVELS (U/L)

MALES

	DOSE LEVEL (ppm)		
Test/Time	0	40	140
3 months			
Alk. Phos	175	465**	500**
SGOT	26	33*	35*
SGPT	19	158**	164**
Sorb.dehyd.	5	14*	20**
6 months			
Alk. Phos.	130	383**	469**
SGOT	24	31*	34**
SGPT	18	158**	154**
Sorb.dehyd.	2	7**	10**
12 months			
Alk.Phos.			
SGOT	23	29	32*
SGPT	15	134**	154**
Sorb.dehyd.	4	11**	13**

* = $p \leq 0.05$

** = $p \leq 0.01$

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Table II (cont.)

FEMALES

Test/Time	DOSE LEVEL (ppm) 0	40	140
3 months			
Alk. Phos	179	503**	615**
SGOT	25	29	41*
SGPT	17	130**	157**
Sorb.dehyd.	6	15**	14**
GGT	5	7*	7*
6 months			
Alk. Phos.	184	415	755**
SGOT	22	25	30*
SGPT	21	121**	145**
Sorb.dehyd.	5	7	6
GGT	5	7	9*
12 months			
Alk.Phos.	152	353	623*
SGOT	24	28	35*
SGPT	17	126**	134**
Sorb.dehyd.	5	132**	10**
GGT	5	7	8

* = $p \leq 0.05$
** = $p \leq 0.01$

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TABLE III
MEAN RBC AND BRAIN CHOLINESTERASE LEVELS (% CONTROL)

MALES Dietary levels (ppm)	0	40	140
RBC month			
3	1405	980(70)	185(13)**
6	1410	1035(73.4)	260(18.4)**
12	1385	1105(70)	315(22.7)**
Brain			
12[a]	2.14	2.04(95.3)	1.57(73.3)
12[b]	1.53	1.52(99.3)	1.29(84.3)

FEMALES Dietary levels (ppm)	0	140
RBC month		
3	1445	245(17)**
6	1240	300(24.1)*
12	1285	310(24.1)*
Brain		
12[a]	2.15	1.68(78.1)
12[b]	1.57	1.31(83.4)

** = $p \leq 0.01$

* = $p \leq 0.05$

a = fractions from vermis and cerebellum

b = right hemisphere minus vermis.

No significant differences in RBC and brain cholinesterase levels were present in other groups which received the test compound.

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Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. Marion P. Copley 9/29/87
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2-year chronic/onco - mouse (83-5)
TOX. CHEM NO: 378B

ACCESSION NUMBERS: 262098-106

TEST MATERIAL: Methidathion

SYNONYMS: Supracide

STUDY NUMBER: IRDC # 382-087

SPONSOR: Ciba-Geigy Corp.

TESTING FACILITY: International Research and Development Corp., Mattawan, Mich.

TITLE OF REPORT: Two year dietary oncogenicity study in mice

AUTHOR(S): E.I. Goldenthal

REPORT ISSUED: 3/7/86

CONCLUSION:
NOEL = 10 ppm (1.6 mg/kg/day)*
LEL = 50 ppm (7.5 mg/kg/day)* based on: (males only) red to orange urine, increase ALT, absolute and relative liver weight, histologic liver and biliary alterations including bile duct hyperplasia, biliary stasis, cholangiofibrosis, gall bladder epithelial hyperplasia, chronic hepatitis; (females only) decreased RBC cholinesterase.
At 100 ppm, in addition to the above, in males - there was increased mortality, increased platelets, leukocytes, AST, ALK and plasma cholinesterase, decreased brain cholinesterase, increased absolute and relative spleen weight and EMH, and decreased hepatic EMH; in females - there was decreased brain cholinesterase, increased histologic liver alterations (see above) and cholecystitis.
Oncogenicity = Males only: Increased hepatocellular adenomas and carcinomas at 100 ppm when considered separately and at 50 and 100 ppm when considered in combination. Although adenomas were statistically increased at all dosage levels this may have been due to an unusually low control incidence.

* Calculated using actual time weighted food consumption averages and body weights.

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MTD: The MTD appears to have been reached in the females (100 ppm based on increased brain and red blood cell cholinesterase as well as non-neoplastic histologic lesions in the liver. The MTD in the males may have been exceeded at 100 ppm based on increased mortality (only after 20 months).

Classification: core-minimum

Special Review Criteria (40 CFR 154.7) To be determined by the TB Peer Review Committee. There is a treatment-related increase in liver tumors in the male mice.

A. MATERIALS:

1. Test compound: Methidathion technical. Description - clear liquid at 60°C, solid when refrigerated, Code #533131 M, Purity - not given.

2. Test animals: Species: mouse, Strain: Chr CD-1, Age: 6 weeks, Weight: males 24-30 gm, females 19-25 gm, Source: Charles River Breeding Labs, Portage, Mich.

B. STUDY DESIGN:

1. Animal assignment - Healthy animals were stratified by weight (approximately 1055 animals per sex were received) then randomly assigned from the various weight groups to the following treatment groups (table from study report) such that their variances were determined heterogeneous by Bartlett's Chi-square test.

GROUP	1		2		3		4		5	
DIETARY LEVEL	0 ppm		3 ppm		10 ppm		50 ppm		100 ppm	
SEX	M	F	M	F	M	F	M	F	M	F
ONCOGENICITY PHASE	50	50	50	50	50	50	50	50	50	50
24-MONTH TERMINAL SACRIFICE[a]	ALL SURVIVORS									
CHRONIC TOXICITY PHASE	120	120	120	120	120	120	120	120	120	120
3 MONTH INTERIM SACRIFICE	20	20	20	20	20	20	20	20	20	20
6 MONTH INTERIM SACRIFICE	20	20	20	20	20	20	20	20	20	20
12 MONTH INTERIM SACRIFICE	20	20	20	20	20	20	20	20	20	20
13 MONTH INTERIM SACRIFICE	20[b]	20[b]	20[b]	20[b]	20[b]	20[b]	20[b]	20[b]	20[b]	20[b]
18 MONTH INTERIM SACRIFICE	40[c]	40[c]	40[c]	40[c]	40[c]	40[c]	40[c]	40[c]	40[c]	40[c]
TOTAL ANIMALS PER GROUP	170	170	170	170	170	170	170	170	170	170

[a]Study was terminated after 23 months by request of Sponsor.
[b]Animals were maintained on control diet for one month after month 12 as recovery animals.
[c]All surviving mice in the chronic toxicity phase were sacrificed.

Prior to initiation, auditory evaluations, detailed observations and body weight measurements were conducted. The study was initiated on October 22, 1982 and was terminated on September 21, 1984.

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2. Diet preparation - Diet was prepared by blending the liquified compound with the food, then it was stored at room temperature. Frequency of preparation was not given. Samples of treated food were analyzed monthly for concentration (duplicate samples). Homogeneity was tested prior to study initiation and at approximately week 60 (duplicate samples from the top, middle and bottom of the blender). Stability of the compound in the diet was tested (in duplicate) after storage at room temperature for 10 days and for up to 7 days under refrigeration.

   Results - The actual compound concentration ranged from 80 to 111 % of the nominal concentration, averaging about 91 % for each treatment group. The mixture appeared to be homogeneous at both sampling time periods. Stability - There was a slight decrease (<10 %) in test article concentration following 4 and 7 days storage at room temperature and under refrigeration.

3. Animals received food (Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The statistical procedures used in this study are attached in Appendix 1. The authors used a 2-tailed tests for all analyses. This is less sensitive than the 1-tailed test usually used by the TB statistics department for most clinical chemistries. They also omitted naming the type of non-parametric rank test that was used.

5. The signed quality assurance statement stated that the study was inspected, findings were reported and the report was reviewed as required by the U.S.E.P.A. Good Laboratory Practice Standards of May 2, 1984.

C. METHODS AND RESULTS:

1. Observations:

   Animals were inspected three times a day on weekdays and twice/day on weekends and holidays for signs of toxicity and mortality.

   Toxicity - The only treatment related observation was dark yellow, orange or red urine, frequently observed in some 50 and 100 ppm male mice. There was no similar finding in the female mice. Other signs, such as alopecia, scabbing, abrasions and palpable masses occurred in all groups.

   Mortality (survival) - An unadjusted analysis of the 2 year (oncogenicity phase) groups, using the Fisher's exact test for pairwise comparisons, shows a significant survival disparity at the high dose (males only) compared with

control. This increased mortality was observed primarily during the last 10 weeks of the study (table 2). These mortality results are consistent with those observed in the chronic toxicity phase of this study.

TABLE 1 Survival at 99 weeks

Dose level (ppm)	Survivors/Number started[1] male	female
0	29/50	22/50
3	30/50	28/50
10	29/50	31/50
50	30/50	25/50
100	16/50*	25/50

[1] Only oncogenicity phase animals are included in this table.
* $p < 0.01$, Fisher's exact test

TABLE 2 Survival - after 1 year[1] (number of male survivors)

Dose (ppm) / Week # N	0 50	3 50	10 50	50 50	100 50
52	50	47	47	49	48
82	37	39	42	38	34
86	34	39	41	36	31
91	34	35	37	32	27
95	33	34	33	31	21
99	29	30	29	30	16

[1] Only oncogenicity phase animals are included in this table.

2. Body weight

Animals were weighed weekly for three months, then monthly for the remainder of the study.

There were no biologically relevant treatment-related effects on body weight (see table 3 taken from the study report).

Table 3 Body weights at 99 weeks

Dose level (ppm)	Mean body weights in grams (% difference from controls) male		female	
0	40	--	36	--
3	39	(-2.5)	37	(+2.7)
10	38	(-5.0)	37	(+2.7)
50	39	(-2.5)	37	(+2.7)
100	40	(0.0)	37	(+2.7)

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3. Food and water consumption and compound intake

Consumption was measured weekly for 3 months, then monthly for the remainder of the study. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Food consumption - As can be seen in table 4 (taken from the study report), there was a very slight decrease in food consumption. In the males it was sporadic occurring primarily during the first year of the study. In the females it lasted throughout the study.

TABLE 4 Food consumption

Dose level (ppm)	Average food consumption in mg/kg/day (% difference from controls) male		female	
0	5.4	--	5.7	--
3	5.3	(-1.9)	5.5	(-3.5)
10	5.2	(-3.7)	5.5	(-3.5)
50	5.2	(-3.7)	5.5	(-3.5)
100	5.2	(-3.7)	5.4	(-5.3)

Food efficiency and water intake - There were no treatment-related effects on either parameter.

Compound Intake - Although compound intake was not discussed in the text, estimates of daily intake using the conversion factor (.15) from the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" were 0.45, 1.5, 7.5 and 15 mg/kg/day for 3, 10, 50 and 100 ppm, respectively. These values are similar to the mean time weighted daily averages (calculated for this DER using the weekly compound intake data): 0.46, 1.6, 7.5 and 16.1 mg/kg/day for 3, 10, 50 and 100 ppm, respectively.

4. a. Ophthalmological examination

Performed on all animals prior to study initiation, and on 50 control and high dose mice per sex at 6, 12 and 18 months.

There were no treatment-related ocular effects.

b. Auditory evaluation - 50 mice per sex from controls and the high dose group were tested for ear twitching using the Galton whistle at 0 and 6 months of study.

This test was discontinued at 6 months because it was determined "the mice were not sensitive to the methodology."

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5. Blood was collected at 3, 6, 12, 13, 18, 24 (weeks 99 and 100) months for hematology and clinical analysis from 10 prefasted randomly selected mice/sex/group. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
X	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

The company reported no treatment-related changes in hematologic parameters. There was however, a significant decrease in HCT (18 %) and HGB (16-19%), and an increase in platelets (40 %) at 12 and 24 months in the 100 ppm males (see table 5). There was also a non-significant increase in platelets at (15-39 %) at the other time points for this group. Leukocytes were increased (44-78 %) at 3, 6, 12 and 18 months (see table 5) in the 100 ppm males. Other results were within expected ranges for this species and strain.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*		Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes		Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
X	Cholinesterase (ChE)#		Triglycerides
	Creatinine phosphokinase*^	X	Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

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As can be seen in table 5, there was an increase in ALK in the 100 ppm males throughout the study and at 24 months in the 50 ppm males. The values at 100 ppm were statistically significant (using non-parametric analysis). Females however, had a significant increase in ALK only at 12 months in the 100 ppm group. ALT was significantly increased in the 100 ppm males throughout the study and at 12, 18 (not significant) and 24 months in the 50 ppm males (see table 5). ALT returned to normal after the 1 month recovery period (13 month study) in the 50 ppm group and almost to normal in the 100 ppm group. AST was also increased from 50 to 140 % (not statistically significant by parametric analysis, significant by non-parametric analysis) in the 100 ppm males (see table 5) at all time points except following treatment withdrawal.

As seen in table 6, plasma cholinesterase values were significantly increased 54 % over control values in the 100 ppm males only. Red cell cholinesterase was significantly decreased 30-45 % in the 100 ppm males and in the 50 and 100 ppm females at most time points. Brain cholinesterase was significantly decreased 22-49 % in the 100 ppm males and females. Sporadic decreases at other treatment levels were probably due to biological variation rather than treatment. The cholinesterase effects were only partially reversed following the 1 month withdrawal from treatment.

6. Urinalysis

Urine was not collected.

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TABLE 5 Select hematology and clinical chemistry values - males

Month	Dose Group (ppm)				
	0	3	10	50	100
Platelets (x 10^3/cmm)					
3	792	737	727	820	956
6	893	825	837	855	1044
12	714	841	665	731	1013**
13[1]	710	643	557	850	990
18	1270	1154	1007	1223	1461
24	1210	1346	1068	1090	1712*
Leukocytes (x 10^3/cmm)					
3	4.3	4.7	3.9	4.2	6.8**
6	3.7	3.8	3.4	4.3	6.6*
12	4.5	4.2	4.0	4.5	7.1*
13[1]	7.9	7.2	4.9	7.1	11.4
18	5.3	7.3	5.4	6.6	8.8*
24	4.3	3.9	4.2	5.3	4.4
Alkaline Phosphatase (IU/L)					
3	37	52	51	35	496
6	47	30	31	71	256
12	42	41	30	29	446
13[1]	45	33	40	51	448
18	49	36	34	64	981
24	32	44	26	88*	265
Aspartate Aminotransferase - AST (IU/L)					
3	108	107	109	89	169
6	89	76	73	81	146
12	76	84	85	86	184
13[1]	96	113	111	86	110
18	110	82	75	111	161
24	83	73	58	118	173
Alanine Aminotransferase - ALT (IU/L)					
3	57	55	51	47	140**
6	55	52	43	77	137*
12	43	48	50	129*	161**
13[1]	55	50	56	45	100
18	58	51	57	104	130**
24	44	35	21	98*	136**

*significantly different from controls at $p<0.05$ (2-tailed)
**significantly different from controls at $p<0.01$ (2-tailed)

[1]These mice had a 1 month recovery period prior to this sample.

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TABLE 6 Cholinesterase values

Month	Dose Group (ppm)	MALE 0	3	10	50	100	FEMALE 0	3	10	50	100
	Plasma Cholinesterase (uM/ml/min)										
3		7.6	7.0	6.4	7.9	9.0	11.7	11.8	11.4	11.4	10.7
6		6.6	7.0	6.7	7.1	8.4*	11.4	11.7	11.7	12.1	10.6
12		7.9	7.4	7.8	7.5	9.3*	11.3	12.6	10.7	12.0	10.4
13[2]		8.5	7.5	8.3	8.3	9.7	11.8	12.0	12.2	12.8	13.3
18		7.0	7.1	7.8	12.1	10.1**	10.7	10.4	11.2	11.6	10.4
24		6.8	7.3	7.3	8.3	10.5**	9.1	9.8	9.6	9.9	8.3
	Red Cell Cholinesterase (uM/ml/min)										
3		2.2	2.3	2.1	1.7	1.2**	2.0	2.1	2.1	1.4*	1.1**
6		3.3	3.2	2.9	2.8	2.2	3.3	3.1	3.2	2.1**	2.2**
12		4.5	4.3	4.1	3.3	2.8*	4.2	4.5	4.4	3.4	2.4**
13[2]		4.2	3.4	3.1	3.5	5.3	2.7	2.8	2.8	2.7	3.1
18		3.3	2.8	3.0	2.4	3.0	2.6	2.6	2.6	1.9*	1.7**
24		3.7	4.4	3.5	2.8	3.0	3.1	3.2	3.1	2.3*	2.2**
	Brain Cholinesterase (uM/g/min)										
3		25.5	27.8	27.3	21.8	15.5**	25.4	25.8	25.0	24.3	19.5**
6		23.5	25.2	23.1	17.9*	14.7**	21.7	23.6	22.2	22.5	16.5*
12		22.4	23.2	22.1	19.5	11.5**	22.2	23.6	21.8	20.3	16.5**
13[2]		26.6	25.9	26.9	24.7	22.5**	25.4	27.7	25.7	25.3	24.2
18		23.8	23.7	25.4	21.4	15.8**	24.5	26.8	25.3	22.0	19.1**
24		24.6	26.7	27.3*	21.0	15.7**	25.3	23.8	23.4	21.2*	16.7**

*significantly different from controls at $p<0.05$ (2-tailed)
**significantly different from controls at $p<0.01$ (2-tailed)

[2]These mice had a 1 month recovery period prior to this sample.

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7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Only control and HDT livers were examined at 3 months. All livers were examined at the 12 and 13 month sacrifices. Animals sacrificed at 24 months, and those from the oncogenicity phase that died on study were subjected to a complete pathologic examination, including histology. Although animals sacrificed at 6 and 18 months and those from the chronic phase that died on study, were examined for macroscopic lesions, they not examined histologically. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Perish. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen	X	Eyes (optic n.)*#
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
X	Colon*	X	Urinary bladder*	X	Mammary gland*#
X	Rectum*	XX	Testes*+	X	Parathyroids*++
XX	Liver *+	X	Epididymides	X	Thyrcids*++
XX	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle	X	Bone*#
Respiratory		XX	Ovaries*+	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
XX	Lung*			X	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight

Treatment-related changes in organ weight included an increase in absolute and relative (percent of body weight) liver weight in the males. Within 3 months, liver weight in the 100 ppm males, was about 50 % greater than controls (significant at $p < 0.01$) increasing to 70%

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by 1 year and more than 100 % at 18 and 24 months (see table 7). This increase was still present after the 1 month withdrawal from treatment. Liver weight in the 50 ppm males was also significantly increased (about 30 %) at 18 and 24 months. There was also a significant increase (67 % over controls) in splenic weight in the 100 ppm males only at 24 months. Sporadic kidney and adrenal weight changes occurred in the females, however they were not related to dose and were not consistent over time.

TABLE 7 Select organ weights in males

Dose Group (ppm)	0	3	10	50	100
Liver at 18 months					
absolute (g)	2.28	2.32	2.46	2.92*	4.76**
relative (% body weight)	5.58	5.72	6.51	7.49**	11.75**
Spleen at 24 months					
absolute (g)	0.12	0.13	0.12	0.14	0.20*
relative (%body weight)	3.02	3.24	3.06	3.45	5.03*

* $p < 0.05$ (2-tailed)
** $p < 0.01$ (2-tailed)

b. Gross pathology

Treatment-related macroscopic lesions were limited to the liver and included enlargement, discoloration, foci, nodules and masses, primarily in the 100 ppm males and to a lesser extent, 50 ppm males and 100 ppm females. Enlarged livers in the 100 ppm males were observed by 3 months. A slight increased incidence of cysts and foci and a moderate increase in discoloration were observed by 6 months in males at 100 ppm. Although there was an increase in masses in all males groups at 12 months, the incidence was slightly higher at 100 ppm. Treatment-related liver lesions in the females were first observed at 12 months and consisted of an increased incidence of discoloration at 100 ppm. Masses, nodules and/or foci were only slightly increased over control levels in both 100 ppm males and females by 18 months. At 24 months, results were similar to 18 months, with the addition of a slight increase of masses in the 50 ppm males and a marked increase of masses and nodules in the 100 ppm males. These results were consistent with the treatment-related changes in organ weight and increases in non-neoplastic (males and females) and neoplastic (males only) alterations described in the following section (C.7.c.) of this review. Although the study report noted

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an increased incidence of generalized splenic enlargement, this only occurred occasionally and was not present at 24 months.

c. Microscopic pathology

1) Non-neoplastic

The primary treatment-related effects were limited to the biliary system and liver. As can be seen in table 8, bile duct hyperplasia, biliary stasis and/or cholangiofibrosis were observed in 95 % of the HDT males within the first 3 months of study. Since the 50 ppm animals sacrificed at 3 months were not examined histologically, it can not be determined if this group was affected at this time period. By 12 months however, 40 and 100 % of the 50 and 100 ppm males, respectively, and 20 and 55 % of the 50 and 100 ppm females, respectively were affected. These lesions were still present at 13 months, following the 1 month withdrawal period from treatment. Similar results were observed at 24 months in the oncogenicity phase. Although there was about a 20 % incidence of these lesions in the 50 ppm females at 12 and 13 months chronic sacrifices, there were no affected mice from 12 to 24 months in the oncogenicity phase. Other related lesions included chronic hepatitis, and gallbladder epithelial hyperplasia in the 50 and 100 ppm males and cholecystitis in the 100 ppm females. There was little to no treatment related affect on areas of cellular alteration and the term hepatocellular hyperplasia was not used in the report. There was also a possible treatment-related decrease in extramedullary hematopoiesis (EMH) noted only in the males at 24 months (5 % at 100 ppm as compared to <30 % in all other groups). In contrast, there was an increase in splenic EMH in the 50 and 100 ppm males, also primarily at 24 months. Myeloid hyperplasia, an observation of normal bone marrow, may have been slightly decreased in incidence and degree in the 100 ppm males at 24 months.

2) Neoplastic

The only treatment related neoplastic changes observed in the oncogenic phase of this study, were increased incidences of <u>hepatocellular adenomas</u> (benign), <u>hepatocellular adenocarcinomas</u> (malignant) and <u>combined</u>, in the males (table 9). Although the first liver tumor was observed at day 445 (in the HDT), the average days until death for males with either of the above liver tumor types were 618, 603, 649, 608 and 608 for the 5 groups, starting with the controls. As can

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be seen in table 9, the combined incidence (adenoma and carcinoma) is significant for both the 50 and 100 ppm

TABLE 8

Bile duct hyperplasia, biliary stasis, and/or cholangiofibrosis (affected/examined)

Dose Group (ppm)	0	3	10	50	100
Males					
Chronic phase					
Sacrificed at:					
3 months	0/20	NE	NE	NE	19/20
12 months	0/20	2/20	1/20	8/20	20/20
13 months	1/19	0/20	0/20	6/20	19/19
Onco phase					
Death at:					
0-12 months	0/0	0/3	0/3	0/1	2/2
12-24 months	1/22	1/18	0/21	12/21	35/36
Sacrifice at:					
24 months	0/28	0/29	0/26	15/28	12/12
Females					
Chronic phase					
Sacrificed at:					
3 months	0/20	NE	NE	NE	1/20
12 months	0/20	0/20	0/20	4/20	11/20
13 months	1/19	1/20	0/20	3/20	10/20
Onco phase					
Death at:					
0-12 months	1/4	0/2	1/3	2/6	1/4
12-24 months	0/27	1/20	3/19	0/20	7/22
Sacrifice at:					
24 months	0/19	1/28	0/28	0/24	4/24

NE - livers not examined histologically

groups, carcinomas are significant only at the high dose and adenomas are significant at all treatment levels. The statistical significance of adenomas however, may be due to an unusually low number of adenomas in the controls (1 out of 50 animals) (see discussion). There is also a positive dose related trend for the two liver tumor types, both individually and in combination. Although these statistics were performed using effective proportions (animals dying prior to the occurrence of the first tumor are censored), the significance is similar when crude proportions (denominator of 50) are used. As can be seen in table 10, there were no significant treatment-related increases in female mouse liver tumors.

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TABLE 9 Number of males in the oncogenic phase with select liver tumors (%[1])

Dose Group (ppm)	0	3	10	50	100
# of livers exam. in onco phase	50	50	50	50	50
Animals on test or day 445[2]	46	44	44	42	43
Adenoma (Ad)	1(2)+	9(20)**	7(16)*	8(19)**	24(56)****
Carcinoma (Ca)	8(17)+	6(14)	4(9)	13(31)	17(40)*
Ad+Ca[3]	9(20)+	15(34)	11(25)	21(50)***	38(88)****

[1] Percent affected, using effective proportions (denominator is the number of animals surviving until day 445.
[2] Day of first observed liver tumor.
[3] Tumor bearing animals counted only once.
* $p < 0.05$ - Fisher's test using effective proportions.
** $p < 0.01$ - Fisher's test using effective proportions.
*** $p < 0.005$ - Fisher's test using effective proportions.
**** $p < 0.001$ - Fisher's test using effective proportions.
+ $p < 0.001$ - Chi-square for linear trend using effective proportions.

TABLE 10 Number of females in the oncogenic phase with select liver tumors (%[1])

Dose Group (ppm)	0	3	10	50	100
# of livers exam. in onco phase	50	50	50	50	50
Adenoma (Ad)	3(6)	9(18)	3(6)	1(2)	3(6)
Adenocarcinoma (Ca)	2(4)	3(6)	2(4)	2(4)	5(10)
Ad+Ca[2]	5(10)	10(20)	5(10)	2(4)	8(16)

[1] Percent affected, using crude proportions (denominator is the total number of animals examined in this phase).
[2] Tumor bearing animals counted only once.

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D. DISCUSSION:

The NOEL for males was 10 ppm and the LEL was 50 ppm based primarily on hepatic and cholinesterase alterations. The NOEL for females, however was 50 ppm and the LEL was 100 ppm based on the same changes. Oncogenic changes, consisting of hepatocellular adenomas and carcinomas occurred only in the males.

The MTD appears to have been reached in the females (100 ppm based on increased brain and red blood cell cholinesterase as well as non-neoplastic histologic lesions in the liver. The MTD in the males may have been exceeded at 100 ppm based on increased mortality (only after 20 months).

The NOEL for clinical signs was 10 ppm and the LEL was 50 ppm based on changes in urine color. There was no speculation as to why this change did not occur in the females. Since urinalysis was not performed, it can not be determined if the color was directly due to a metabolite, parent or a result of systemic toxicity causing elimination of pigments such as hemoglobin, myoglobin or bilirubin.

The NOEL for mortality was 50 ppm and the LEL was 100 ppm based on decreased survival in the males after 20 months. Body weight, food efficiency and water consumption were not affected by treatment. The slight changes that were noted for food consumption were probably due to individual animal variation. There were no treatment-related ophthalmologic effects.

The NOEL for hematology was 50 ppm and the LEL was 100 ppm based on increased platelet and leukocyte counts in the 100 ppm males. These may have been an inflammatory response to generalized liver and biliary toxicity or liver tumors. Although the slight decreases in HCT and HGB (100 ppm males) were consistent with other alterations in the hematopoietic system (discussed later) they were most likely a result of individual animal variation since the values were within the expected range for this species.

The NOEL for clinical chemistry was 10 ppm and the LEL was 50 ppm based on increased ALT in the male. This was evident in the 100 ppm males by 3 months and 12 months in the 50 ppm males. There was also a treatment-related increase in AST and ALK in the 100 ppm males by 3 months. The sporadic ALK increases in the 50 ppm males at 24 months and 12 month females (100 ppm) were probably unrelated to treatment.

The NOEL for cholinesterase (Che) was 10 ppm and the LEL was 50 ppm based on red cell cholinesterase inhibition in the females. Red cell cholinesterase was also decreased in the

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HDT males. Brain cholinesterase was inhibited in both males and females at the HDT. There was an increase in plasma cholinesterase in the HDT males, however the significance of this in unknown.

The NOEL for organ weights was 10 ppm and the LEL was 50 ppm, based on increased absolute and relative liver weight. The author reported that increased absolute and relative liver weights at 18 months in the 50 ppm males was not treatment-related because there were no associated macroscopic changes. These increases however, occurred at both 18 and 24 months and macroscopic liver changes were noted at 24 months. Although there was no microscopic analysis at 18 months, minimal microscopic changes were present in the 50 ppm group at 12 months. These became more prominent by 24 months (including liver tumors). Therefore, the increased liver weight observed in the 50 ppm males was probably related to treatment. The increased splenic weight at 24 months in the HDT males is consistent with increased EMH noted histologically.

The NOEL for non-neoplastic histologic lesions was 10 ppm and the LEL was 50 ppm based on hepatic and biliary changes in the males, including bile duct epithelial hyperplasia, biliary stasis, cholangiofibrosis, gall bladder hyperplasia, chronic hepatitis and cholecystitis. The lesions were more severe, and occurred at lower doses in the males than in females. These changes were consistent with the increased liver weights and clinical chemistry changes. Hepatic changes in the 12 and 13 month 50 ppm females (chronic phase) were probably not treatment-related since they were not present in the oncogenic phase of the study at 12 and 24 months. There was a possible bone marrow depression (mentioned by the author but not supported in the study's results and discussion sections) as indicated by decreased myeloid hyperplasia. However, this could not be confirmed without additional information from a bone marrow smear and M:E:ratio. The decreased EMH in the liver, a normal hematopoietic organ in the mouse, may be a result of generalized hepatotoxicity and neoplasia. The spleen, also a normal hematopoietic organ in the mouse, had an increase in EMH over control levels, with a corresponding increase in organ weight. This suggests a possible response to either blood loss or decrease in normal RBC production (ie. bone marrow depression).

This compound causes an increase in hepatic tumors in male mice. Although adenomas appear significantly increased at all treatment levels, it is probably due, in all but the high dose, to an unusually low control incidence (2%). The control number of carcinomas appears to be unusually high (17%). Historical control values (see Appendix 2, submitted

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by the registrant) for studies conducted in this laboratory between 1978 and 1983 are:

Liver tumors	mean % from 11 studies (range)
adenomas	11 (0 - 26.7)
carcinomas	5.7 (0 - 14.3)
combined (benign + malignant) animals counted only once	16.7 (5 - 26.7)

Although this is old historical control data (newer data has been requested from the registrant), more current data will not significantly alter the conclusions of the oncogenic potential of this chemical. The 100 ppm group is the only one with a statistical increase in carcinomas. When both benign and malignant tumors are combined, there is a statistical increase at both 50 and 100 ppm.

There were many deficiencies in this report. The results and discussion text are incomplete and are not always consistent with the initial summary. For example, observations (ie. bone marrow depression) made in the initial summary of the study are not supported (or refuted) in the results and discussion making the report difficult to follow. Although individual animal and summary data tables were present, there were no results and discussion of the 3, 6 and 12 month sacrifice and pathology results. The table on page 40, entitled "Summary of test article related neoplastic changes in male mice" had 3 HDT tumor bearing animals counted twice in the combined incidence row. Animals containing both hepatocellular adenomas and carcinomas should only be counted once. There were no urinalysis tests conducted in this study. Results from these tests may have assisted in determining the cause of colored urine. Two-tailed tests were used rather than the more conservative 1-tailed tests used by the TB statistics department for most clinical chemistries. Therefore, treatment-related effects could not be determined based primarily on significance. The report did not state what non-parametric rank test was used and what the underlying distribution assumption for the test was. However, since the above mentioned deficiencies do not alter the conclusions from this study, it is classified as core-minimum for both chronic and oncogenicity and satisfies the guideline requirement for both (guideline 83-5).

Questions to the company:

1. What non-parametric rank test was used for the clinical chemistries (ie. ALK) and
2. what underlying distribution assumption was made for the non-parametric rank test used?

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Appendix I

V. STATISTICS

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A. METHODS

1. Randomization Procedure

At the end of the pretest period, each animal number and the corresponding body weight were entered in a magnetic-disc data base which was used as the data source for the computer calculated randomization procedure.

The mean body weight for all animals of the appropriate sex in the quarantine population was computed, after which the absolute difference from the quarantine population mean weight was computed for each animal, and the animals sorted in order of increasing absolute difference from the population mean. After the initial sorting, the appropriate number of blocks of animals was designated and the required number of animals was assigned to treatment groups by randomizing each successive block of animals. Bartlett's Chi-square test for homogeniety of variances was performed on these groups. If the group variances were judged heterogeneous, new randomizations were generated until homogeneity was established at which time the randomization was accepted.

2. Data Analysis

Body weight and food consumption (weekly for the first 13 weeks, then monthly thereafter), food efficiency (weeks 1-13), water consumption (monthly for the first 3 months, then once approximately every 3 months thereafter), hematology, biochemistry and cholinesterase values (3, 6, 12, 13 and 18 months and at study termination) and absolute and relative organ weight data (3, 6, 12, 13 and 18 months and at study termination) were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel and Torrie[1] and Ostle[2].

Dunnett's[3] multiple comparison tables were used to determine significance.

Gamma glutamyltranspeptidase, chloride, alkaline phosphatase and aspartate aminotransferase were analyzed using a nonparametric approach, by transforming the data to ranks prior to analysis, as described by Conover and Iman[4]. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance. Survival data and data on time to neoplasm were analyzed using the computer program of Thomas, Breslow and Garc[5]. Statistical procedures included in this program are the Kaplan-Meier and standard methods for computing survival curves, Cox's test for linear trend in proportions and both Cox's test and Gehan-Breslow's generalized Kruskal-Wallis test for comparing survival distributions. Data on time to neoplastic lesion were analyzed for all benign tumors, all malignant tumors, all tumors combined, all mortalities and for each individual tumor type that appeared in two or more animals in the high-dose group.

B. RESULTS

The results of the data analyses are presented in the appropriate sections of the report.

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Approved And Submitted by:

Emma C. Kalra, M.S.A.
Director of Statistics and Data Processing

2/14/86
Date

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
HISTORICAL CONTROL TUMOR DATA - LIVER
2-YEAR CHARLES RIVER CD-1 MOUSE STUDIES

STUDY	A		B		C		D		E		F		G		H		I		J		K		AVE		MIN		MAX	
SEX	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
NUMBER OF ANIMALS ON STUDY	75	75	60	60	60	60	60	60	50	50	80	80	60	60	70	70	100	100	60	60	50	50						
TERMINAL NECROPSY	7/78		7/82		2/80		7/83		7/83		11/82		11/82		8/81		12/81		10/81		1/82							
BENIGN TUMORS																												
HEPATOCELLULAR ADENOMA																												
Incidence			4				16	2	12	2	15	2	13	1	2	2	12	3	3		3	1						
% Incidence			6.7				26.7	3.3	24.0	4.0	18.8	2.5	21.7	1.7	2.9	2.9	12.0	3.0	5.0		6.0	2.0	11.0	1.8	0	0	26.7	4.0
HEMANGIOMA																												
Incidence				1			2										1	2		2								
% Incidence				1.7			3.3										1.0	2.0		3.3			0.4	0.7	0	0	3.3	3.3
TOTAL BENIGN TUMORS																												
Incidence			4	1			18	2	12	2	15	2	13	1	2	2	13	5	3	2	3	1						
% Incidence			6.7	1.7			30.0	3.3	24.0	4.0	18.8	2.5	21.7	1.7	2.9	2.9	13.0	5.0	5.0	3.3	6.0	2.0	11.4	2.5	0	0	30.0	5.0
MALIGNANT TUMORS																												
HEPATOCELLULAR CARCINOMA																												
Incidence	4	1			3						5				10	2	11	1	5	1	3							
% Incidence	5.3	1.3			5.0						6.3				14.3	2.9	11.0	1.0	8.3	1.7	6.0		5.7	0.7	0	0	14.3	2.9
HEMAGIOSARCOMA																												
Incidence		1									1	1			1							2						
% Incidence		1.3									1.3	1.3			1.4							4.0	0.3	0.5	0	0	1.4	4.0
TOTAL MALIGNANT TUMORS																												
Incidence	4	2			3						6	1			11	2	11	1	5	1	3	2						
% Incidence	5.3	2.7			5.0						7.5	1.3			15.7	2.9	11.0	1.0	8.3	1.7	6.0	4.0	5.9	1.2	0	0	15.7	2.9
TOTAL BENIGN AND MALIGNANT TUMORS																												
Incidence	4	2	4	1	3		18	2	12	2	21	3	13	1	13	4	24	6	8	3	6	3						
% Incidence	5.3	2.7	6.7	1.7	5.0		30.0	3.3	24.0	4.0	26.3	3.8	21.7	1.7	18.6	5.7	24.0	6.0	13.3	5.0	12.0	6.0	17.4	3.7	5.0	0	30.0	6.0

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Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 7/29/87
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/22/87
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2 generation repro. - rat (83-4) TOX. CHEM NO:378B

MRID NUMBER: 400798-12, -13

TEST MATERIAL: Methidathion

STUDY NUMBER: 450-2125

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: American Biogenics Corporation, Decatur, Il.

TITLE OF REPORT: Two-generation reproduction study in rats.

AUTHOR: Clare Salamon

REPORT ISSUED: January 15, 1986

CONCLUSIONS:

Parental Systemic Toxicity NOEL = 5 ppm
LEL = 25 ppm based on tremors and decreased food consumption during lactation, and decreased ovarian weight (relative and absolute). In addition to the above, at the HDT there was a slight decrease in body weight during lactation and a transient decrease in body weight early in the F_1 growth phase (males and females).

Reproductive NOEL = 5 ppm
LEL = 25 ppm based on a decreased mating index and a generalized indication of pup unthriftyness while nursing, characterized by decreased pup weight and an increased incidence of hypothermia with the appearance of starvation. In addition to the above, at the HDT there was an increase in stillbirths and decreased pup survival at birth and during lactation.

Classification: core-minimum

Special Review Criteria (40 CFR 154.7) Not triggered by this study.

A. MATERIALS:

1. Test compound: Technical methidathion, Description - crystalline solid at room T°, lot # - FL-841649, Purity - not given. The test material was stored frozen.

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2. Test animals: Species: rats, Strain: Charles River (CRl:CD BR) Sprague-Dawley, Age: 30 days at start of study, Weight (range): male - 74-127 gm, females - 51-127 gm, Source: Charles River, Portage, Mi., Animals were acclimated for 1 week prior to test. Animals were individually housed, except during mating and lactation, in environmentally controlled rooms.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly (by computer), to test groups (see table 1). Rats were fed the appropriate test diet throughout the entire study.

TABLE 1

Test Group	Conc. in diet ppm	F_0 (parents of f_1 pups) male	F_0 (parents of f_1 pups) female	F_1 (parents of f_2 pups) male	F_1 (parents of f_2 pups) female
1 Cont	0	15	30	15	30
2 Low (LDT)	5	15	30	15	30
3 Mid (MDT)	25	15	30	15	30
4 High (HDT)	50	15	30	15	30

The approximate schedule from the study report is in attachment 1. The F_0 generation parents were maintained on the test diet for 12 weeks prior to breeding (about 16 weeks of age) for the f_1 litters. These progeny were raised until weaning age (Postpartum (PP) day 21). The F_1 pups were maintained on the test diet for 12 weeks prior to breeding (about 16 weeks of age) for the f2 litters.

2. Mating procedure

Males were bred to 2 females, each for a period of 10 days. The females were checked daily for copulatory plugs and vaginal spermatozoa. Females with confirmed matings were then housed individually. If mating did not occur, the female was mated to a different male (maximum of 2 mating attempts).

3. Diet preparation

Test diet was prepared weekly and stored at room temperature in closed containers until used. Aliquots of test material were melted at 45-50°C then dispersed in the food. Diet was tested for homogeneity and chemical stability prior to study initiation. Concentration of the diets was tested monthly.

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pregestation and gestation - There were no treatment-related clinical signs of toxicity.
lactation - Muscle tremors were observed in the 15/20 of the 50 ppm group dams, primarily during the second and third week of lactation. Tremors were not observed in the 25 ppm group dams.

TABLE 2 Alopecia (Female)(affected/treated)

	dose (ppm)			
	0	5	25	50
F_0	3/30	4/30	7/30	12/30
F_1	2/30	0/30	2/30	1/30

2. Body weight

Animals were weighed weekly for 12 weeks prior to mating. Females were weighed on gestation days (G) 0, 7, 14, and 20; and those with litters were also weighed on PP 0, 7, 14, and 21 (during lactation). Females without viable progeny and all males were weighed monthly until sacrifice.

F_0 - male - No treatment-related changes in body weight.
female -
pregestation and gestation - No treatment-related changes in body weight.
lactation - Body weights in the HDT dams were between 10 - 20 % lower than controls during the second and third weeks of lactation (significant at the 95% CL[1]) (see attachment 2).

F_1 - male - Initial body weights (weaning weight) at the HDT were about 30% lower (significant at the 99 % CL) than controls and remained about 10 % lower (at the 95 % CL) for the first two months. There was a non-significant decrease at the MDT for the first month (about 10 %).
female -
pregestation - Initial body weight (weaning weight) was decreased 14 and 29 % (significant at the 95 and 99 % CL, respectively) in the MDT and HDT females. Body weights were similar to control levels for the remainder of this period.
gestation - No treatment-related changes in body weight.
lactation - There was a 10 % decrease (significant at the 95 % CL) in body weight during the second and third weeks of lactation in the HDT dams (see attachment 2).

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[1] CL - confidence limit

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3. Food consumption, food efficiency and compound intake

Food consumption was determined weekly for 12 weeks prior to mating. It was measured on gestation days (G) 0, 7, 14, and 20; and for those with litters on PP 0, 7, 14, and 21 (during lactation). Food efficiency was not presented in the report. Compound intake was calculated.

Food consumption -

F_0 - male - There were no treatment-related changes in food consumption.
female -
pregestation - There were no treatment-related changes in food consumption.
gestation - There were no treatment-related changes in food consumption.
lactation - There was a slight (10 %) decrease in consumption (HDT) during the second and third weeks (significant at the 95 % CL only for week 3).

F_1 - male - There was a 10-14 % (usually significant at the 95 % CL) decrease in food consumption at 25 ppm (weeks 1 and 2) and at 50 ppm (weeks 1 through 3).
female -
pregestation - There were no treatment-related changes in food consumption.
gestation - There were no treatment-related changes in food consumption.
lactation - There was a 26 % and 19 % decrease in consumption (HDT) during the second and third weeks, respectively (significant at the 99 and 95 % CL, respectively).

Compound intake within each group was fairly consistent for each time interval. There was the expected decrease in intake/kg of body weight, as the animals gained weight. The time weighted average and total compound intake were not reported.

4. Reproductive effects

Sex, weight and number of live and dead progeny (and partially cannibalized) were determined on the day of delivery (PP0).

Mating index - (# of copulations/# of estrus cycles) X100
Fertility index - (# of pregnancies/# of copulations) X100
Gestation index - (# of parturitions/# of pregnancies) X100
Female fertility index -
(# of pregnancies/# of females mated) X100
Male fertility index - (# of sires/# of males mated) X100

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Results of mating performance and fertility -

F_0 - As can be seen in attachment 3 (table 12 of the study report), a significant decrease in the fertility index and female fertility index occurred only in the 25 ppm group.

F_1 - There was a decrease in mating index in both the 25 and 50 ppm groups (see attachment 3). There was evidence of copulation for all 15 males in every group (both generations) with at least 1 female.

Delivery data - f_{1a} and f_{2a} litters - There was a non-significant increase in stillbirths at 50 ppm and a decrease in viable pups at birth (see attachment 4, table 13 from the study report). As can be seen in attachment 5 (table 15 from the study report) there was a slight, but significant decrease (about 3 %) in pup birth weight (PP0) at the MDT and HDT. All other delivery parameters were similar to control values.

5. Lactation effects (Progeny measurements)

Pups were examined at birth and at weaning for gross developmental anomalies. Surviving neonates were examined twice daily and counted on days PP4, 7, 14 and 21. On day PP4, the litters were randomly culled to 8 pups, 4 males and 4 females when possible. Individual pup weights were obtained on days PP0, 4, 7, 14 and 21.

Neonatal survival (presented in attachment 4) for the 50 ppm f_{1a} pups was only slightly decreased (see table 3), however in the f_{2a} 50 ppm litters, viability was significantly depressed (70 % of the viable young survived through day PP4) (see attachment 4). By PP day 21, only 66 % of the PP day 4 pups survived.

TABLE 3 % Viability during lactation

test group (ppm)	Lactation day PP 4[a]	PP 7[b]	PP 14[b]	PP 21[b]
f_{1a} litters				
0	98	100	100	100
5	97	99	98	97
25	95	92	91	89
50	86	97	92	90
f_{2a} litters				
0	96	100	100	99
5	94	99	98	98
25	94	99	99	98
50	70	85	69	66

[a] surviving pups/viable pups at birth
[b] surviving pups/pups retained after culling on day PP 4

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Pup body weight (see attachment 5) was significantly decreased in a dose related-manner at both 25 and 50 ppm throughout lactation. The only treatment-related observations during lactation in both the MDT and HDT were increased incidences of pups that were cool to touch and appeared starving (table 4).

TABLE 4 Select observations [% pups affected (% litters affected)]

	dose (ppm)							
	f_{1a}				f_{2a}			
	0	5	25	50	0	5	25	50
cool	0(0)	0(0)	8.6(10.5)	12.0(30.4)	0(0)	0(0)	0.7(8.3)	2.7(23.8)
starved	0(0)	0(0)	12.9(15.8)	3.8(8.7)	0(0)	0(0)	0(0)	11.2(23.8)

6. Sacrifice and Pathology

Parents - Complete necropsy was performed and tissues (liver, ovaries, prostate, seminal vesicles, coagulating gland, testes, uterus, vagina, cervix, pituitary gland, abnormal tissues) were fixed for histologic examination. In addition, the brain (including brain stem), liver, and ovaries or testes (with epididymides) were weighed. Animals that were found dead were given a gross external and internal examination (no organs were weighed).
F_0 - The males were sacrificed at 195 days of age and the females at 196-7 days having been on test diet for approximately 165 and 166-7 days, respectively.
F_1 - The males were sacrificed at 167-183 days of age and the females at 168-189 days.

Progeny -
Progeny that died during lactation (<21 days old) were given a gross external and internal examination.
Progeny that were culled were not examined.
f_{1a} and f_{2a} pups - Ten randomly selected (computerized procedure) male and female pups from each group were given a gross external and internal examination. Although the period of time between weaning and this necropsy was not given, it appeared to be about 1 week. It also could not be determined whether the weanlings were given test diet during this interval. Brain (including brain stem), liver, and ovaries or testes (with epididymides) were weighed. Selected tissues (see parents) were fixed, however only abnormal tissues were examined histologically. All pups found dead during lactation and those with abnormal developmental anomalies were also examined grossly. The remaining pups were sacrificed without an examination.

a. Organ weights (parents) - There was a treatment related decrease (about 22 %) in relative and absolute ovary

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weight when compared to controls, in both the F_0 (MDT and HDT) and F_1 (HDT) females (see table 5). There was also a 6 % decrease (significant at the 99 % CL) in absolute brain weight in the F_0 HDT females. However there was no corresponding decrease in relative brain weight.

TABLE 5 Ovary weight

	F_0				F_1			
dose (ppm)	0	5	25	50	0	5	25	50
absolute (g)	.0991	.1023	.0827	.0776*	.1134	.1138	.1036	.0910*
relative (g/100g BW)	.0306	.0312	.0238*	.0229**	.0351	.0349	.0322	.0283*

* different at the 95 % CL
** different at the 99 % CL

Organ weights (pups) - There were no organ weight changes in the f_{1a} pups. In the f_{2a} pups there was a decrease in absolute brain, liver and testes weight without a corresponding decrease in relative organ weight.

b. Gross and microscopic pathology - Parental and progeny - There were no apparent treatment-related gross or microscopic lesions.

D. DISCUSSION

The NOEL for parental systemic toxicity was 5 ppm (LDT) and the LEL was 25 ppm (MDT) based on tremors and decreased food consumption in the females, during lactation. There was also a transient decreased food consumption in the mid and high dose F_1 males early in the feeding phase. This however, was probably a carry-over from their general unthriftyness during lactation rather than a direct effect of treatment during this phase. The significance of the decreased absolute and relative ovarian weights at the mid (F_0) and high (F_0 and F_1) is unclear since there were no corresponding histologic ovarian changes. There were no treatment related deaths (parents). Overt clinical signs of toxicity were limited to tremors during lactation at the mid (F_0) and high (F_0 and F_1) doses. It is possible that they were due to the combination of cholinesterase inhibition from methidathion and stress of lactation, since tremors were not observed in males or females during pregestation and gestation. The slight increase in alopecia observed in the mid and high dose F_0 females may be treatment related, however it was only observed in the F_0 females during the feeding phase of the study. Other effects of treatment observed only at the high dose, included a slight (but statistically significant) decrease in body weight during lactation in the females (both breeding trials). Initial body

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weights in the F_1 parental males and females were also decreased from controls. This, however, was a result of poor weight gain while nursing. This is consistent with the transient decrease in food consumption discussed previously.

The NOEL for perinatal reproductive effects was 5 ppm (LDT) and the LEL was 25 ppm (MDT) based on a decreased mating index. Since all males copulated with at least 1 female, this decrease appears to be a female problem. In addition, at the HDT there were slight, but non-significant decreases in viability at birth and in birth weight as well as a non-significant increase in stillbirths, all probably related to treatment. The decreased fertility index in the F_0 mating was probably not treatment-related since there was no corresponding change at the HDT or in the F_2 mating.

The NOEL for lactation reproductive effects was 5 ppm (LDT) and the LEL was 25 ppm (MDT) based on a generalized indication of pup unthriftyness during nursing, characterized by decreased pup weight and an increased incidence of hypothermia with the appearance of starvation. Pup survival was affected primarily at the HDT. The decreased absolute organ weights in the HDT pups were probably a function of decreased body weight, rather than due to treatment since there was no corresponding decrease in relative organ weights. There is no indication whether these lactation effects were a result of poor milk production or toxicity from the compound in the milk.

The study procedures were often difficult to follow, and in certain cases, important facts were omitted from the report. For example, it could not be determined how long after weaning the 10/sex/group f_{1a} pups were sacrificed and what they were fed during this interval. As mentioned above, only 10 pups/sex/group were necropsied and then, only grossly abnormal tissues were histologically examined. However, it is not expected that these deficiencies would alter the conclusions since it appears (based on body weight) that less than 1 week elapsed prior to the sacrifice of the pups, and all of the F_0 and F_1 parents had complete necropsies including histopathology. These results are consistent with those from a preliminary study (450-1713) completed just prior to this study. This study is therefore classified as core-minimum and satisfies the FIFRA requirement for a 2 generation reproduction study.

NOTE: The Office of Compliance Monitoring (OCM) submitted a Good Laboratory Practice investigation report concerning this study to the National Laboratory Audit Program. At the time Toxicology Branch (TB) received this report, the study had not yet been submitted for review (see TB memorandum dated 5/5/86 from M. Copley to L. Schnaubelt). The primary issue in the report concerned homogeneity in the diet due to the use of very small quantities of liquid. Minor issues included quality assurance, computer security, preparation of adequate SOPs and feed

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contamination with vermin. As mentioned in the earlier TB memo, these latter concerns appeared to have no significant effect on the study results. The homogeneity concern also appears to be resolved, at least in part by the study report. The diet/compound variability did not appear to be significant, although the sampling procedures were not listed in detail. There was little variation between the monthly samples. Of greater concern to TB was the stability of the compound in the diet. The loss in activity was however, only about 15 % after 1 week. This should have no major impact on the conclusions since there were clear cut effects at the MDT and HDT and a clear NOEL at the LDT.

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ATTACHMENT 1

Selection of

First (F0) Generation — F0 Generation Parental Animals

Pre-mating period (12 weeks)

F1a mating trials (3 weeks)

Gestation periods (3 weeks from mating)

Lactation periods (3 weeks from delivery)

F1a weanings

- Pathology
- Second (F1) Generation

F0 parent sacrifice

Second (F1) Generation — F1 Generation Parental Animals

Pre-mating period (12 weeks)
F2a mating trials (3 weeks)
Gestation periods (3 weeks from mating)
Lactation periods (3 weeks from delivery)
F2a weanings
- Pathology

F1 parent sacrifice

Approximate durations are given in parentheses.

4. Statistical Analyses

Parental body weights and food consumption data, progeny population and survival data, and progeny body weight data were analyzed using Analysis of Variance. Significant differences between the untreated control group and the treated groups were evaluated using an appropriate multiple comparison test (Tukey's or Scheffe's dependent upon 'N' values). Progeny body weights were further analyzed using Analysis of Covariance and Dunnett's T-test with the litter size as the covariate.

Organ weight ratios were studied using Kruskal-Wallis analyses. Chi-Square analysis and Fisher's Exact Tests were performed where appropriate.

All statistical analyses were interpreted using the untreated control group for comparison. Differences were considered significant at the $p<0.05$ and $p<0.01$ confidence limits.

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ATTACHMENT 2

MATERNAL BODY WEIGHT DATA

TWO GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1A LITTER

GROUP (PPM)		BODY WEIGHT (GM) MEAN VALUES GESTATION DAY: 0	7	14	20	LACTATION DAY: 0	4	7	14	21
U-C (0.0)	MEAN	287.	314.	344.	401.	317.	319.	324.	353.	345.
	S.D.	31.1	34.9	42.5	42.6	35.5	34.9	36.4	34.0	37.2
	N	28	28	28	28	28	28	28	28	28
T-I (5.0)	MEAN	293.	324.	354.	409.	325.	333.	335.	360.	341.
	S.D.	35.2	40.9	47.4	50.3	41.8	43.5	40.4	36.0	37.3
	N	25	25	25	25	25	24	24	24	24
T-II (25.0)	MEAN	297.	328.	356.	420.	327.	326.	324.	340.	328.
	S.D.	37.3	41.2	45.8	44.6	43.9	42.8	35.3	32.5	31.9
	N	18	18	18	18	19	19	18	18	18
T-III (50.0)	MEAN	295.	325.	353.	412.	329.	326.	328.	324.*	312.*
	S.D.	28.8	30.2	28.7	33.2	28.8	28.0	27.2	27.5	27.3
	N	23	23	23	23	23	22	22	22 8%	21 10%

F1 GENERATION - F2A LITTER

GROUP (PPM)		BODY WEIGHT (GM) MEAN VALUES GESTATION DAY: 0	7	14	20	LACTATION DAY: 0	4	7	14	21
U-C (0.0)	MEAN	279.	306.	330.	391.	308.	313.	317.	336.	316.
	S.D.	29.9	30.8	30.9	35.8	34.2	30.6	29.9	29.8	26.2
	N	25	25	25	25	25	25	25	25	25
T-I (5.0)	MEAN	283.	316.	339.	399.	314.	321.	325.	345.	324.
	S.D.	33.8	37.5	42.5	49.8	41.2	37.8	34.5	37.1	29.4
	N	29	29	29	29	29	28	28	28	28
T-II (25.0)	MEAN	292.	317.	343.	394.	310.	308.	309.	320.	305.
	S.D.	60.8	60.6	60.6	60.4	46.9	41.7	40.3	35.4	32.1
	N	26	26	26	26	24	24	24	24	24
T-III (50.0)	MEAN	281.	303.	327.	375.	300.	293.	299.	296.**	283.*
	S.D.	46.5	45.7	48.0	51.9	44.4	36.7	40.3	34.0	37.8
	N	22	22	22	22	21	18	16	14 12%	14 10%

S.D. = STANDARD DEVIATION
N = NUMBER OF ANIMALS
*STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95% CONFIDENCE LEVEL
**STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99% CONFIDENCE LEVEL

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ATTACHMENT 3

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REPRODUCTIVE PERFORMANCE

TWO-GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1A LITTER

GROUP (PPM)	MATING INDEX RATIO	PERCENT	FERTILITY INDEX RATIO	PERCENT	GESTATION INDEX RATIO	PERCENT	FEMALE FERTILITY INDEX RATIO	PERCENT	MALE FERTILITY INDEX RATIO	PERCENT	AVERAGE GESTATION LENGTH (DAYS)
U-C (0.0)	30/41	73.2	28/30	93.3	28/28	100.0	28/30	93.3	15/15	100.0	22
T-I (5.0)	30/50	60.0	25/30	83.3	25/25	100.0	25/30	83.3	13/14	92.9	22
T-II (25.0)	30/46	65.2	19**/30	63.3	19/19	100.0	19**/30	63.3	12/14	85.7	22
T-III (50.0)	28/46	60.9	23/28	82.1	23/23	100.0	23/30	76.7	14/15	93.3	22

**STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99% CONFIDENCE LEVEL

F1 GENERATION - F2A LITTER

GROUP (PPM)	MATING INDEX RATIO	PERCENT	FERTILITY INDEX RATIO	PERCENT	GESTATION INDEX RATIO	PERCENT	FEMALE FERTILITY INDEX RATIO	PERCENT	MALE FERTILITY INDEX RATIO	PERCENT	AVERAGE GESTATION LENGTH (DAYS)
U-C (0.0)	29/33	87.9	25/29	86.2	25/25	100.0	25/29	86.2	14/15	93.3	22
T-I (5.0)	30/37	81.1	29/30	96.7	29/29	100.0	29/30	96.7	15/15	100.0	22
T-II (25.0)	29*/42	69.0	27/29	93.1	24/27	88.9	27/29	93.1	15/15	100.0	22
T-III (50.0)	29*/44	65.9	23/29	79.3	22/23	95.7	23/30	76.7	13/15	86.7	22

*STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95% CONFIDENCE LEVEL

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ATTACHMENT 4

DELIVERY AND POPULATION DATA

TWO GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1A LITTER

NUMBER OF PROGENY:

GROUP (PPM)		DELIVERED TOTAL	DELIVERED MEAN	VIABLE TOTAL	VIABLE MEAN	STILLBORN TOTAL	STILLBORN MEAN	CANNIBALIZED TOTAL	CANNIBALIZED MEAN
U-C (0.0)		368	13.1	365	13.0	3	0.1	0	0.0
	S.D.		3.41		3.38		0.31		
	N		28		28		28		28
T-I (5.0)		333	13.3	330	13.2	0	0.0	3	0.1
	S.D.		3.63		3.66				0.44
	N		25		25		25		25
T-II (25.0)		260	13.7	255	13.4	5	0.3	0	0.0
	S.D.		3.07		2.99		0.73		
	N		19		19		19		19
T-III (50.0)		304	13.2	292	12.7	12	0.5	0	0.0
	S.D.		3.29		2.98		0.95		
	N		23		23		23		23

F0 GENERATION - F1A LITTER

NUMBER OF VIABLE PROGENY
LACTATION DAY:

GROUP (PPM)		4 TOTAL	4 MEAN	RETAINED 4 TOTAL	RETAINED 4 MEAN	7 TOTAL	7 MEAN	14 TOTAL	14 MEAN	21 TOTAL	21 MEAN
U-C (0.0)		356	12.7	217	7.8	217	7.8	217	7.8	217	7.8
	S.D.		3.18		0.97		0.97		0.97		0.97
	N		28		28		28		28		28
T-I (5.0)		321	12.8	192	7.7	191	7.6	188	7.5	187	7.5
	S.D.		3.76		1.60		1.60		1.61		1.64
	N		25		25		25		25		25
T-II (25.0)		243	12.8	149	7.8	137	7.2	135	7.1	133	7.0
	S.D.		3.05		0.69		1.99		2.08		2.16
	N		19		19		19		19		19
T-III (50.0)		252	11.0	169	7.3	164	7.1	156	6.8	152	6.6
	S.D.		4.15		1.85		1.94		2.07		2.39
	N		23		23		23		23		23

S.D. = STANDARD DEVIATION
N = NUMBER OF ANIMALS
NO STATISTICALLY SIGNIFICANT DIFFERENCES NOTED

F1 GENERATION - F2A LITTER

NUMBER OF VIABLE PROGENY
LACTATION DAY:

GROUP (PPM)		4 TOTAL	4 MEAN	RETAINED 4 TOTAL	RETAINED 4 MEAN	7 TOTAL	7 MEAN	14 TOTAL	14 MEAN	21 TOTAL	21 MEAN
U-C (0.0)		303	12.1	193	7.7	193	7.7	193	7.7	192	7.7
	S.D.		3.21		0.89		0.89		0.89		0.90
	N		25		25		25		25		25
T-I (5.0)		354	12.2	220	7.6	218	7.5	216	7.4	216	7.4
	S.D.		3.54		1.64		1.66		1.70		1.70
	N		29		29		29		29		29
T-II (25.0)		294	12.3	186	7.8	185	7.7	185	7.7	182	7.6
	S.D.		2.95		0.85		0.86		0.86		0.93
	N		24		24		24		24		24
T-III (50.0)		186	8.5AA	137	6.2	116	5.3AA	95	4.3AA	90	4.1AA
	S.D.		4.71		3.13		3.44		3.48		3.50
	N		22		22		22		22		22

F1 GENERATION - F2A LITTER

NUMBER OF PROGENY:

GROUP (PPM)		DELIVERED TOTAL	DELIVERED MEAN	VIABLE TOTAL	VIABLE MEAN	STILLBORN TOTAL	STILLBORN MEAN	CANNIBALIZED TOTAL	CANNIBALIZED MEAN
U-C (0.0)		315	12.6	312	12.5	3	0.1	0	0.0
	S.D.		3.40		3.45		0.44		
	N		25		25		25		25
T-I (5.0)		375	12.9	367	12.7	0	0.3	0	0.0
	S.D.		3.64		3.43		0.80		
	N		29		29		29		29
T-II (25.0)		313	13.0	305	12.7	7	0.3	1	0.0
	S.D.		3.11		3.18		0.91		0.20
	N		24		24		24		24
T-III (50.0)		267	12.1	259	11.8	8	0.4	0	0.0
	S.D.		3.24		3.75		0.95		
	N		22		22		22		22

S.D. = STANDARD DEVIATION
N = NUMBER OF ANIMALS
NO STATISTICALLY SIGNIFICANT DIFFERENCES NOTED

ATTACHMENT 5

BODY WEIGHT DATA - PROGENY

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TWO GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1A LITTER

GROUP (PPM)		PROGENY BODY WEIGHT(GM) MEAN VALUES LACTATION DAY: 0	4	7	14	21 MALE	21 FEMALE
U-C (0.0)	MEAN	6.2	9.4	15.6	28.5	47.3	44.4
	S.D.	0.9	2.1	3.3	5.5	9.2	8.1
	N	365	356	217	217	108	109
T-I (5.0)	MEAN	6.2	9.8	15.9	29.2	47.5	44.8
	S.D.	0.7	1.7	2.5	4.4	7.1	7.1
	N	330	321	191	188	92	95
T-II (25.0)	MEAN	6.3	9.0	14.3**	25.3**(A)	40.0**	40.6*
	S.D.	0.8	2.2	3.6	5.6	9.1	9.0
	N	255	243	137	135	69	64
T-III (50.0)	MEAN	6.0	8.6**(A)	13.0**(B)	21.4**(B)	33.4**(B)	31.2**(B)
	S.D.	0.9	2.1	3.4	4.7	8.7	8.1
	N	292	252	164	156	76	76

F1 GENERATION - F2A LITTER

GROUP (PPM)		PROGENY BODY WEIGHT(GM) MEAN VALUES LACTATION DAY: 0	4	7	14	21 MALE	21 FEMALE
U-C (0.0)	MEAN	6.1	9.5	15.4	27.9	46.0	43.9
	S.D.	0.7	1.5	2.3	3.7	6.6	6.0
	N	312	303	193	193	96	96
T-I (5.0)	MEAN	6.1	9.2	14.9	28.2	47.5	44.7
	S.D.	0.7	1.5	2.6	3.8	6.2	6.4
	N	367	354	218	216	105	111
T-II (25.0)	MEAN	5.9*	8.6**	13.6**(A)	23.9**(B)	39.2**	35.1**(A)
	S.D.	0.8	1.7	2.8	4.9	9.5	7.3
	N	305	294	185	185	90	92
T-III (50.0)	MEAN	5.9**	7.4**(B)	10.6**(B)	19.1**(B)	28.4**(B)	26.2**(B)
	S.D.	0.8	1.6	2.9	4.4	8.4	5.5
	N	259	186	116	95	49	41

S.D. = STANDARD DEVIATION
N = NUMBER OF ANIMALS
*STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95% CONFIDENCE LEVEL USING ANALYSIS OF VARIANCE AND SCHEEFE'S MULTIPLE COMPARISON (INDIVIDUAL PUP BODY WEIGHT DATA)
**STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99% CONFIDENCE LEVEL USING ANALYSIS OF VARIANCE AND SCHEEFE'S MULTIPLE COMPARISON (INDIVIDUAL PUP BODY WEIGHT DATA)
(A)STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95% CONFIDENCE LEVEL USING ANALYSIS OF COVARIANCE (LITTER SIZE AS THE COVARIATE) AND DUNNETT'S T-TEST (MEAN LITTER WEIGHT DATA)
(B)STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99% CONFIDENCE LEVEL USING ANALYSIS OF COVARIANCE (LITTER SIZE AS THE COVARIATE) AND DUNNETT'S T-TEST (MEAN LITTER WEIGHT DATA)

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Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. Marion P. Copley 7/20/87
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/22/87
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2 generation repro. - rat (83-4) TOX. CHEM NO:378B

MRID NUMBER: 400798-11

TEST MATERIAL: Methidathion

STUDY NUMBER: 450-1713

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: American Biogenics Corporation, Decatur, Il.

TITLE OF REPORT: Two-generation reproduction study in rats.

AUTHOR: Clare Salamon

REPORT ISSUED: June 13, 1986

CONCLUSIONS after 1 generation:
Parental Systemic Toxicity NOEL = 5 ppm
LEL = 50 ppm based on muscle tremors and decreased food consumption in the females during the lactation period. In addition to the above, at the HDT there was decreased body weight, weight gain and food consumption (during pregestation).
Reproductive NOEL = 5 ppm
LEL = 50 ppm based on decreased pup survival and weight (PP0-21). At the HDT there were also tremors in the pups, decreased pup, viability as well as a decreased mating index.

Classification: core-supplementary; study was terminated after one generation due to extreme toxicity at the high dose.
Special Review Criteria (40 CFR 154.7) Not ~~triggered~~.

A. MATERIALS:

1. Test compound: Technical methidathion, Description - crystalline solid at room T°, lot # - FL-841649, Purity - not given. The test material was stored frozen.

2. Test animals: Species: rats, Strain: Charles River CD Sprague-Dawley, Age: 4 weeks at start of study, Weight - not specified, Source: Charles River, Portage, Mi., Animals were acclimated for 1 week prior to test. Animals were individually housed, except during mating and lactation, in environmentally controlled rooms.

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B. STUDY DESIGN:

1. Animal assignment

Animals were randomly assigned by computer, to test groups (see table 1). Rats were fed the appropriate test diet throughout the entire study.

TABLE 1

Test Group	Conc. in diet ppm	F_0 (parents of f_1 pups) male	female
1 Cont	0	15	30
2 Low (LDT)	5	15	30
3 Mid (MDT)	50	15	30
4 High (HDT)	100*	15	30

* Concentration was changed to 25 ppm at weaning of f_{1a} litters until study was terminated.

Approximate schedule from the study report is in attachment 1. Rats in the F_0 generation were maintained on the test diet for 12 weeks prior to mating (about 16 weeks of age). They were bred to obtain the f_{1a} litters. These progeny were raised until weaning age (Postpartum (PP) day 21). The F_0 rats were then bred following a 2 week rest period, producing the f_{1b} litters. The study was terminated prior to delivery of all these litters due to poor conception.

2. Mating procedure

Males were bred to 2 females, each for a period of 10 days. The females were checked daily for copulatory plugs and vaginal spermatozoa. Females with confirmed matings were then housed individually. If mating did not occur, the female was mated to a different male (maximum of 2 mating attempts). Females were mated with a different male for each mating. Females that failed to conceive during the first breeding trial were not rebred.

3. Diet preparation

Test diet was prepared weekly and stored at room temperature in closed containers until used. Aliquots of test material were melted at 45-50°C then dispersed in the food. Diet was tested for homogeneity and chemical stability prior to study initiation. Concentration of the diets was tested monthly.

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Results - Homogeneity and stability - Samples from the top middle and bottom of the mixing vessel were sampled and checked for homogeneity. It could not be determined whether the values presented were means or the only sample. There did not appear to be any significant difference due to sampling location. The stability test indicated a decrease in compound concentration in the diet after 7 days of about 13 to 16 %. Monthly repeated sampling indicated that the concentrations remained fairly consistent (within 8 % of nominal) throughout the study.

4. Animals received food (Purina Certified Rodent Chow No, 5002) and water ad libitum.

5. Statistics - The statistical procedures (taken from the study report) used for analyzing the numerical data are in attachment 1.

6. A signed quality assurance statement was included with the report.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality. A more detailed examination was conducted weekly.

Mortality - There were no treatment-related deaths in the F_0 rats.

Toxicity - Pregestation (growth phase), mating, and gestation - There were no treatment-related signs of toxicity in either males or females.
Lactation - Muscle tremors were observed in the 18/21 of the 100 ppm group dams early in lactation. Pups from these dams subsequently died. Tremors were also observed in 6/21 of the 50 ppm group dams, however these were not evident until the third week of lactation.

2. Body weight

Animals were weighed weekly for 12 weeks prior to mating. Females were weighed on gestation days (G) 0, 7, 14, and 20; and those with litters were also weighed on PP 0, 7, 14, and 21 (during lactation). Weight gains were not reported. Females without viable progeny and all males were weighed monthly until sacrifice.

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Pregestation - As can be seen in table 2, there was a significant decrease in body weight at 100 ppm for males and females. Body weight gain during this period was decreased in the males and females only during the first month of treatment.
Gestation and lactation - Body weight ($p<0.05$ and $p<0.01$, for gestation and lactation, respectively) and weight gain were decreased during gestation and lactation in the HDT group females. Although the MDT females also weighed less (body weight minus gravid uterus) during lactation, their body weights were not significantly different from control values due to marked variation within the group. Subsequent to decreasing the 100 ppm diet to 25 ppm, male and female body weight in this group, returned to control levels.

TABLE 2 Selected body weights (gm) and weight gains

Test Group (ppm)	males (F_0) weight w 10	males (F_0) weight m 5	females (F_0) weight w 10	females (F_0) weight m 5	females (F_0) weight gain f_{1a} preg.	females (F_0) weight gain f_{1a} lact
0	487.0	609.5	272.1	323.1	104	10
5	477.5	611.1	274.0	326.2	108	5
50	465.9	598.6	283.0	345.0	98	-25
100^	445.3*	579.8^	247.1**	296.1^	80	-12

w = week
m = month
^ concentration was decreased to 25 ppm at 5 months (after lact of f_{1a} litters).
* statistically significant difference, at 95 % CL.
** statistically significant difference, at 99 % CL.

3. Food consumption, food efficiency and compound intake

Food consumption was determined weekly for 12 weeks prior to mating. It was measured on gestation days (G) 0, 7, 14, and 20; and for those with litters on days PP 0, 7, 14, and 21 (during lactation). Food efficiency was not presented in the report. Compound intake was calculated using the mean daily food consumption, individual body weight and theoretical dietary concentration.

Food consumption - Pregestation - Food consumption in the HDT males and females, was significantly decreased only during the first 2 weeks of treatment (12 % less than controls). Although the male's food consumption remained similar to controls for the remainder of the study, the HDT female's consumption was frequently statistically increased as much as 20 % over control levels.

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Gestation and lactation - Food consumption during gestation was similar to controls. During the first 2 weeks of lactation, it was ($p<0.01$) decreased as much as 34 % in the HDT group. In the MDT it was also decreased ($p<0.01$) about 20 % during the second week.

Food efficiency, although not calculated in the report, appeared to be unaffected in the males and decreased in the females for much of the pregestation period.

Compound intake within each group was fairly consistent for each time interval. There was the expected decrease in intake/kg of body weight, as the animals gained weight. The time weighted average and total compound intake were not reported.

4. Reproductive effects

Sex and number of live and dead progeny were (and partially cannibalized) determined on the day of delivery (PP).

Mating index - (# of copulations/# of estrus cycles) X100

Fertility index - (# of pregnancies/# of copulations) X100

Gestation index - (# of parturitions/# of pregnancies) X100

Female fertility index -
(# of pregnancies/# of females mated) X100

Male fertility index - (# of sires/# of males mated) X100

Results of mating performance and fertility - Only the mating index for the 100 ppm group (for the f_{1a} mating) was statistically less than controls ($p<0.05$)(see table 3). The mating index for this group during the f_{1b} mating, (test concentration had been decreased to 25 ppm) was also significantly less ($p<0.01$) than controls. All other indices were similar to controls.

TABLE 3 Mating index

Test Group (ppm)	f1a	f1b
0	76.9	63.6
5	59.2	64.1
50	58.3	54.5
100^	51.0*	27.5**^

^ concentration was decreased to 25 ppm at 5 months (after lact. of f_{1a} litters, 2 weeks prior to the f_{1b} mating trial).
* statistically significant difference, at 95 % CL.
** statistically significant difference, at 99 % CL.

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Delivery data - f1a and f1b litters - There was a significant decrease (table 4) in pup birth weight (PP0) at the MDT and HDT. As can be seen in attachment 2 (table 15 taken from the study report), there was a non-statistical treatment-related decrease in percent viable pups, and a corresponding increase in percent dead in the 100 ppm group.

5. Lactation effects (Progeny measurements)

Pups were examined at birth and at weaning for gross developmental anomalies. Surviving neonates were examined twice daily and counted on days PP4, 7, 14 and 21. On day PP4 the litters were randomly culled to 8 pups, 4 males and 4 females when possible. Individual pup weights were obtained on days PP0, 4, 7, 14 and 21.

Neonatal survival in the HDT was significantly depressed for f_{1a} (f_{1b} was terminated) litters (57 % of the viable young survived through day PP4)(see attachment 2). Deaths occurred primarily during the first two weeks of lactation. By PP day 21, only 57.4 % of the PP day 4 pups survived (see attachment 2). There was also a slight (12-18 %), but non-significant, decrease in survival during lactation at the MDT. Pup body weight (see table 4) was significantly decreased at both 50 and 100 ppm. The only treatment-related observation during lactation were muscle tremors involving all pups (duration 6 days) in two 100 ppm litters and 1 pup in a third HDT litter (duration 1 day).

TABLE 4 Selected mean pup body weights (gm)

Test Group (ppm)	PP day 0	PP day 14	PP day 21	
			male	female
0	6.3	30.0	52.1	46.9
5	6.3	30.9	53.2	49.0
50	6.0**	23.8**	39.0**	35.4**
100	5.6**	19.9**	30.5**	30.1**

** statistically significant difference, at 99 % CL.

6. Sacrifice and Pathology

F_0 (parents) - These rats were sacrificed and discarded at 223 days of age due to problems with the study. F_0 animals that were found dead were given a gross external and internal examination.

Progeny -
Progeny that died during lactation (<21 days old) were given a gross external and internal examination.
Progeny that were culled were not examined.

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f_{1a} pups - Ten randomly selected (computerized procedure) male and female pups from each group were given a gross external and internal examination. The period of time between weaning and this necropsy was not given. It could also not be determined whether the weanlings were given test diet or control diet. Brain (including brain stem), liver, and ovaries or testes (with epididymides) were weighed. Selected tissues were fixed for possible histopathologic studies. Pups with any abnormal developmental anomalies were also examined grossly. The remaining pups were sacrificed without an examination.
f_{1b} pups - All were sacrificed and discarded at weaning (21 days of age).

a. Organ weights (pups) - There was a significant decrease in absolute but not relative liver and testes weights at the high dose. There was also an increase in relative but not absolute brain weight. Unfortunately it could not be determined how long after weaning these pups were sacrificed, and what they were fed during this interval.

b. Gross pathology - Parental - F_0 parents were not examined.
Progeny - There were no treatment-related external anomalies.

D. DISCUSSION

The NOEL for parental systemic toxicity was 5 ppm (LDT) and the LEL was 50 ppm (MDT) based on tremors and decreased food consumption in the females, during lactation. Clinical signs of toxicity in the F_0 parental generation were limited to treatment and dose-related tremors during lactation at the mid and high doses. It is possible that they were due to the combination of cholinesterase inhibition from methidathion and stress of lactation, since tremors were not observed in males or females during pregestation and gestation. There was an earlier onset of these tremors and a higher frequency observed at the high dose as compared to the mid-dose. Other effects of treatment included decreased body weight in the HDT males and females until the dose was decreased to 25 ppm. The decreased weight gain occurred primarily during the first month of the feeding phase. Gain was again decreased during gestation and lactation in the HDT females. The decreased body weight, observed during lactation in the MDT females, did not appear to be treatment-related, since it was not significantly different from controls and there was marked variation within this group. During the growth phase there was an treatment-related decrease in food consumption (first two weeks only) at the HDT (males and females). Subsequently, there was a periodic increase in consumption in the HDT females. This may have been a result of decreases feed efficiency since there was no corresponding

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increase in body weight when compared to controls. During gestation, there was no effect on consumption, however it was decreased during lactation in both the MDT and HDT females.

The NOEL for perinatal reproductive effects was 5 ppm (LDT) and the LEL was 50 ppm (MDT) based on decreased pup birth weight. A decreased mating index and a slight decrease in pup viability were observed at the HDT. Although decreased body weight appeared reversible when the HDT dose (100 ppm) was decreased to 25 ppm prior to breeding for the f_{1b} litters, the mating index was actually lower than during the first mating.

The NOEL for lactation reproductive effects was 5 ppm (LDT) and the LEL was 50 ppm (MDT) based on decreased pup weight. Pup survival was affected primarily at the HDT with a lesser but treatment-related decrease also at the MDT. There were tremors noted in some HDT pups as well. The altered organ weights in the HDT male and female pups were probably a function of decreased body weight, rather than due to treatment.

It is likely that 100 ppm exceeded the MTD, as suggested by the author, since the mating index, pup survival and pup weight were severely affected.

The study procedures were often difficult to follow, and in certain cases, important facts were omitted from the report. For example, it could not be determined how long after weaning the 10/sex/group f_{1a} pups were sacrificed and what they were fed during this interval. This study was terminated after only one generation due to severe toxicity at the high dose tested, therefore this study is classified as core-supplementary.

NOTE: The Office of Compliance Monitoring (OCM) submitted a Good Laboratory Practice investigation report concerning this study to the National Laboratory Audit Program. At the time Toxicology Branch (TB) received this report, the study had not yet been submitted for review (see TB memorandum dated 5/5/86 from M. Copley to L. Schnaubelt). The primary issue in the report concerned homogeneity in the diet due to the use of very small quantities of liquid. Minor issues included quality assurance, computer security, preparation of adequate SOPs and feed contamination with vermin. As mentioned in the earlier TB memo, these latter concerns appeared to have no significant effect on the study results. The homogeniety concern also appears to be resolved, at least in part by the study report. The diet/compound variability did not appear to be significant, although the sampling procedures were not listed in detail. There was little variation between the monthly samples. Of greater concern to TB was the stability of the compound in the diet. The loss in activity was however, only about 15 % after 1 week. This should have no major impact on the conclusions since there were clear cut effects at the MDT and HDT and a clear NOEL

at the LDT.

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ATTACHMENT 1

FLOW CHART

1st Generation — Selection of F0 Generation Parental Animals

- Pre-mating period (12 weeks)
- F1a mating trials (3 weeks)
- Gestation periods (3 weeks from mating)
- Lactation periods (3 weeks from delivery)
- F1a weanings
- Rest period (2 weeks)
- F1b mating trials (3 weeks)
- Gestation periods (3 weeks from mating)
- Lactation periods
- Study terminated (May 7, 1985)

Approximate durations are given in parentheses.

4. Statistical Analyses: Parental body weights and food consumption data, progeny population and survival data, and progeny body weight data were analyzed using analysis of variance. Significant differences between the untreated control group and the treated groups were evaluated using an appropriate multiple comparison tests (Tukey's or Scheffe's dependent upon 'N' values). Progeny body weights were further analyzed using analysis of covariance and Dunnett's T-test with the litter size as the covariate.

Organ weight ratios were studied using Kruskal-Wallis analyses. Chi-Square analysis and Fisher's Exact Tests were performed where appropriate.

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ATTACHMENT 2

TOTAL PROGENY SURVIVAL

TWO-GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1A LITTER

GROUP (PPM)	PERCENT OF PROGENY BORN: VIABLE	DEAD	CANNI-BALIZED	PROGENY SURVIVAL (PERCENT) LACTATION DAY: 4	7	14	21
U-C (0.0)	99.6	0.4	0.0	97.7	100.0	100.0	98.8
T-I (5.0)	100.0	0.0	0.0	95.4	95.7	95.1	95.1
T-II (50.0)	95.9	4.1	0.0	80.4	96.6	87.7	85.6
T-III (100.0)	94.1	5.5	0.5	57.8**	79.2**	57.4**	57.4**

NOTE: STATISTICAL EVALUATIONS WERE CONDUCTED USING THE INDIVIDUAL LITTER SURVIVAL DATA.
INDICES PRESENTED IN THIS TABLE ARE PROGENY SURVIVAL AS A GROUP.
**STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99% CONFIDENCE LEVEL

TWO-GENERATION REPRODUCTION STUDY IN ALBINO RATS
TEST ARTICLE: METHIDATHION TECHNICAL
F0 GENERATION - F1B LITTER

GROUP (PPM)	PERCENT OF PROGENY BORN: VIABLE	DEAD	CANNI-BALIZED	PROGENY SURVIVAL (PERCENT) LACTATION DAY: 4	7	14	21
U-C (0.0)	98.6	1.4	0.0	100.0	STUDY TERMINATED		
T-I (5.0)	98.8	1.2	0.0	98.2	STUDY TERMINATED		
T-II (50.0)	99.2	0.8	0.0	86.3	STUDY TERMINATED		
T-III (25.0)	90.0	10.0	0.0	98.3	STUDY TERMINATED		

*DOSE LEVEL DURING PARENTAL REST PERIOD AND F1B LITTER (I.E., MATING TRIALS GESTATION PERIOD, AND LACTATION PERIOD, WHERE APPLICABLE)
NOTE: STATISTICAL EVALUATIONS WERE CONDUCTED USING THE INDIVIDUAL PROGENY SURVIVAL DATA.
INDICES PRESENTED IN THIS TABLE ARE THE PROGENY SURVIVAL AS A GROUP.
NO STATISTICALLY SIGNIFICANT DIFFERENCES NOTED
DATA THROUGH MAY 6, 1985; STUDY TERMINATED MAY 7, 1985

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Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 8/12
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 8/27/87
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratology - rabbit (83-3) TOX. CHEM NO: 378B

MRID NO.: 400798-09,10

TEST MATERIAL: Methidathion

SYNONYMS: Supracide

STUDY NUMBER: 86131 (pilot - MIN 852223)

SPONSOR: Ciba-Geigy Corp.

TESTING FACILITY: Ciba-Geigy Corp., Summit, N.J.

TITLE OF REPORT: A teratology (segment II) study in rabbits

AUTHOR: M. Giknis

REPORT ISSUED: January 13, 1987

CONCLUSION:

Maternal toxic NOEL = 6 mg/kg/day.
Maternal toxic LEL = 12 mg/kg/day based on clinical signs typical of cholinesterase inhibition (ataxia, tremors, salivation), miosis, and blood in the pan all of which only occurred during the treatment period.

Developmental NOEL $\geq$ 12 mg/kg/day (HDT).
Developmental LEL was not reached in this study.

A/D ratio (maternal NOEL/develop. NOEL) = 6/12 = 0.5

Classification: core-minimum

Special Review Criteria (40 CFR 154.7) There are no special review triggers in this study.

A. MATERIALS:

1. Test compound: Technical methidathion, Description - Amber liquid/solid (dependent on T°), batch # - 533131M202444, Purity - not given, stable for 24 hours at room T° in vehicle and for 20 days at 6°C.
   Vehicle: 3% aqueous cornstarch with 0.5 % Tween 80. 006385
2. Test animals: Species: rabbit, Strain: New Zealand White

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S.P.F., Age: sexually mature (virgin), Weight (range): males - not given, female - 3.26-4.75 Kg, Source: H.A.R.E.-Marland, Hewitt, N.J. They were given food (Purina certified rabbit chow) and water ad libitum, individually housed in an environmentally controlled room and acclimated for 5 weeks prior to artificial insemination.

B. STUDY DESIGN:

1. Animal assignment

Nineteen artificially inseminated females were randomly assigned to 4 test groups. The day of insemination was called day 0 of gestation (0G).

3. Treatment - Female rabbits were dosed by gavage on days 7G through 19G with one of the following doses: 0, 2, 6, and 12 mg/kg/day. Doses were based on body weight of days 7G, 10G or 14G. They were sacrificed on day 29G. The test solution was prepared twice during the study. Actual concentrations of the test solutions were between 98 and 106 % of the nominal doses with the exception of the first half of the LDT treatment period. Analysis of this concentration indicated that it was only 11 % of nominal concentration. There was no explanation given for this deviation.

4. Statistics - The statistical procedures used in analyzing the numerical data are attached.

5. A signed and dated quality assurance statement was attached to the report.

C. METHODS AND RESULTS:

1. Observations

The rabbits were observed twice daily for toxicity and mortality.

Mortality included 1 MDT (dosing accident) and 1 HDT (broken back). Three does were sacrificed early after they spontaneously aborted (one each from the LDT, MDT and HDT). Treatment-related signs of toxicity (statistically significant at $p \leq 0.01$) were limited to the HDT and included ataxia (4/19), tremors (4/19), salivation (4/19) all of which only occurred intermittently during the treatment period. Miosis and blood in the pan were not statistically significant but occurred in 2/19 and 1/19 does respectively.

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2. Body weight

Rabbits were weighed on days 0G, 7G, 10G, 14G, 20G, 24G and 29G, and weight gain calculated.
There were no treatment-related changes in body weight and weight gain.

3. Food consumption

Food consumption was measured daily from day 6G throughout pregnancy.

There were no treatment-related changes in food consumption.

4. Sacrifice and necropsy examination - Surviving does were euthanized on day 29G (of presumed gestation) with CT-61 solution and any gross abnormalities noted and fixed in formalin. The uterus (with contents) were weighed for all rabbits either at the time of death or at sacrifice.

There were no treatment-related lesions observed at necropsy.

5. Reproductive effects

a. Maternal - The uterus was examined for number and position of live, dead and resorbed fetuses and corpora lutea. Post implantation loss was calculated. The report does not mention whether uteri without visible implantation sites were stained with ammonium sulfide to detect very early resorptions. Ovaries were examined for number of corpora lutea.

There were no treatment-related effects on any maternal reproductive parameters.

b. Fetal

External alterations - All viable fetuses were individually weighed, sexed and examined for gross abnormalities(use of magnification was not mentioned). All fetuses were then placed in 95 % ethanol.
Visceral alterations - All live fetuses were then sexed and examined for visceral abnormalities using a modification of Staples' technique (Staples, 1974).
Skeletal alterations - All fetuses were then stained with Alizarin Red S for skeletal examination using appropriate magnification. Ossification centers were checked for presence or absence, size, shape location and relationship to adjacent ossification centers (method was described but not referenced).

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There were no treatment-related effects on sex ratio or fetal weight. There were no treatment-related external, visceral, or skeletal alterations or variations.

D. DISCUSSION:

The NOEL for maternal toxicity was 6 mg/kg/day and the LEL for maternal toxicity was 12 mg/kg/day based on clinical signs typical of cholinesterase inhibition (ataxia, tremors, salivation), miosis, and blood in the pan, all of which only occurred during the treatment period. The two early deaths (MDT and HDT) were not related to treatment. The NOEL for developmental toxicity was greater than 12 mg/kg/day since an LEL for developmental toxicity was not reached.

Although the purity of the test compound was not given, the % a.i. for the same batch used in another study was 94.1-95.9%. The LDT rabbits appeared to get only 11 % of the nominal dose for half of the treatment period. However, this would not alter the results and conclusions since the MDT was the NOEL and adverse signs would not have been expected at 2 mg/kg/day. Therefore the study is classified as core-minimum.

NOTE: The registrant performed the pilot range-finding study #MIN 852223 (dated 2/25/86) using the following doses: 0, 10, 30 and 50 mg/kg/day given on days 7 through 19G of gestation (there were 6 does/group). There was severe maternal toxicity at the MDT and HDT (100 % mortality in both groups). Signs of maternal toxicity in the 10 mg/kg/day group included: 1/6 deaths and a possible decrease in weight gain. There was an increase in stool variation in does of the LDT group. There were no treatment-related changes in fetal weight or external observations. Further fetal examinations were not conducted.

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2.14. Statistics: Statistical analyses of the data were performed as indicated below or as indicated in the individual reports from Research Statistics:

Statistical Analysis and References

Parametric Analysis On:	Body Weight, Body Weight Gain and Feed Consumption
Statistical Methods:	Bartlett's Test for Homogeneity of Variance*
	For homogeneous Variances - One-Way Analysis of Variance* With Dunnett's Method of Multiple Comparisons
Parametric Analysis On:	Fetal Weight
Statistical Methods:	Healy Analysis
Nonparametric Analysis On:	Number of corpora lutea, implantations, viable fetuses, dead fetuses, resorptions and % post-implantation loss.
Statistical Methods:	Refer to the Individual Statistics Report - Statistical Analysis of Reproductive Parameters in a Teratology Study (Segment II)

*NOTE: If the Bartlett's Test for Homogeneity of Variance produced a probability of $p \leq 0.001$, additional analysis for that parameter/interval was not performed. It was the opinion of the Sponsor that the sensitivity of the statistical analysis that was performed was appropriate for this study and did not jeopardize the study outcome.

Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. 8/27/87 006385
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. 8/27/87
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratology - rat (83-3) TOX. CHEM NO: 378B

MRID NO.: 400798-07,08

TEST MATERIAL: Methidathion

SYNONYMS: Supracide

STUDY NUMBER: 86172 (pilot - 86028)

SPONSOR: Ciba-Geigy Corp.

TESTING FACILITY: Ciba-Geigy Corp., Summit, N.J.

TITLE OF REPORT: A teratology (segment II) study in rats

AUTHOR: R. Infurna

REPORT ISSUED: January 15, 1987

CONCLUSION:

Maternal toxic NOEL = 1.00 mg/kg/day.
Maternal toxic LEL = 2.25 mg/kg/day based on decreased body weight and food consumption during the treatment period and clinical signs typical of cholinesterase inhibition (lethargy, tremors, salivation, lacrimation), exophthalmia, raspy respiration and vaginal bleeding. One dam died.

Developmental NOEL $\geq$ 2.25 mg/kg/day (HDT).
Developmental LEL was not reached in this study.
A/D ratio (maternal NOEL/develop. NOEL) = 1.00/2.25 = 0.44

Classification: core-minimum

Special Review Criteria (40 CFR 154.7) There are no special review triggers in this study.

A. MATERIALS:

1. Test compound: Technical methidathion, Description - Amber liquid/solid (dependent on T°), batch # - 533131M202444, Purity - 94.1-95.9 %, stable for 24 hours at room T° in vehicle and for 20 days at 6°C. 006385
   Vehicle: 3% aqueous cornstarch with 0.5 % Tween 80.
2. Test animals: Species: rat, Strain: Crl:COBS CD (SD)BR,

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Age: sexually mature (virgin), Weight (range): males - not given, female - 203-249 gm, Source: Charles River Labs, Kingston, NY. They were given food (Purina #5002 certified chow) and water ad libitum, and individually housed in an environmentally controlled room.

B. STUDY DESIGN:

1. Animal assignment

Thirty females were randomly assigned to 4 test groups. A maximum of 25 pregnant rats (positive vaginal washing) were continued on study.

2. Mating was considered successful if sperm were found in the vaginal washing. The day of mating was called day 0 of gestation (0G).

3. Treatment - Female rats (maximum of 25/group) were dosed by gavage on days 6G through 15G with one of the following doses: 0 (vehicle), 0.25, 1.0, and 2.25 mg/kg/day. Doses were based on the body weight of days 6G, 8G or 12G. Rats were sacrificed on day 20G. Frequency and method of preparing the test solution were not mentioned. Actual concentrations of the test solutions were between 98 and 104 % of the nominal doses.

4. Statistics - The statistical procedures used in analyzing the numerical data are attached.

5. A signed and dated quality assurance statement was attached to the report.

C. METHODS AND RESULTS:

1. Observations

The rats were observed twice daily for toxicity and mortality.

One HDT dam died (day 12G). Treatment-related signs of toxicity were limited to the HDT and included lethargy (24/25), tremors (25/25), salivation (12/25), lacrimation (4/25), exophthalmia (10/25), raspy respiration (3/25), and vaginal bleeding (4/25) as compared to 0/25 in controls for all signs. These signs occurred intermittently throughout most of the treatment period and stopped after cessation of treatment.

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2. Body weight

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Rats were weighed on days 0G, 6G, 8G, 12G, 16G and 20G, and weight gain calculated.

As can be seen in table 1, body weight gain was decreased (significantly) at the HDT by about 25 % throughout the treatment period and returned to control levels following cessation of treatment.

TABLE 1 Body weight change

Dose level (mg/kg/day)	N±	Ave. body weight change (g/rat) 0-6G	6-16G [trt period]	16-20G	Net gain (minus uterus)
0	24	36.25	57.88	51.04	67.83
.25	18	34.44	56.72	51.61	68.33
1.00	21	33.38	55.71	50.86	67.81
2.25	22	34.22	43.50*	56.59	57.13*

± Number of surviving pregnant dams.
* Significantly different from control, at $p \leq 0.05$

3. Food consumption

Food consumption was measured for the period from days 0G to 6G, then daily throughout pregnancy.

As can be seen in table 2, food consumption was decreased (about 8 to 10 %) only in the HDT group during much of the treatment period. This decrease was significant on days 9G and 12G through 16G. Following cessation of treatment (day 15G) food consumption returned to control levels.

TABLE 2 Food consumption (gm/rat/day)

Dose level (mg/kg/day)	N±	0-6G	6-9G [treatment period]	9-15G	15-20G
0	24	23.08	23.75	25.34	26.75
.25	18	22.84	23.43	24.76	26.31
1.00	21	22.83	24.01	25.68	26.52
2.25	23	23.40	22.67	22.35*	25.87

± Number of surviving pregnant dams.
* Different from controls ($p \leq 0.05$) for most of this period.

4. Sacrifice and necropsy examination - A laparohysterectomy was performed on day 20G following asphyxiation with carbon dioxide. The uterus (with contents) were weighed for all rats, either at the time of death or at sacrifice. Lesions observed at necropsy were limited to hollow or

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fluid filled kidneys (1/25 controls) and fused placenta (1/25 HDT). There were no treatment-related lesions.

5. Reproductive effects

a. Maternal - The uterus was examined for number and position of live, dead and resorbed (early and late) fetuses and corpora lutea. Post implantation loss was calculated. The report does not mention whether uteri without visible implantation sites were stained with ammonium sulfide to detect very early resorptions. Ovaries were examined for number of corpora lutea.

There were no treatment-related effects on any maternal reproductive parameters.

b. Fetal

External alterations - All viable fetuses were individually weighed, sexed and examined for (use of magnification was not mentioned) external alterations.
Visceral alterations - about half of the live fetuses from each litter were fixed (in Bouin's solution) for visceral examination (method of Monie, Kho and Morgan - 1965).
Skeletal alterations - The remaining half of each litter were fixed in 95 % ethanol and processed for skeletal examination (method of Staples and Schnell - 1964).

There were no treatment-related effects on the sex ratio or fetal weight. There were no treatment-related external, visceral, and skeletal alterations or variations.

D. DISCUSSION:

The NOEL for maternal toxicity was 1.00 mg/kg/day and the LEL for maternal toxicity was 2.25 mg/kg/day based on decreased body weight and food consumption during the treatment period, clinical signs typical of cholinesterase inhibition (lethargy, tremors, salivation, lacrimation), exophthalmia, raspy respiration and vaginal bleeding. Although there were no deaths in the pilot study at 7.5 mg/kg/day, the one HDT maternal death was probably treatment-related since the dam had other signs of organophosphate toxicity prior to death on day 12G. The NOEL for developmental toxicity was greater than 2.25 mg/kg/day since an LEL for developmental toxicity was not reached.

NOTE: The registrant performed the pilot range-finding study #86028 (dated 7/22/86) using the following doses: 0, 0.1, 1.0 and 7.5 mg/kg/day given on days 6 through 15 of

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gestation (there were approximately 24 pregnant dams/group). Signs of severe maternal toxicity occurred at the HDT in up to 75 % of the dams. They included: ataxia, chromodacryorrhea, crusty eyes, labored respiration, lacrimation, salivation, tremors, unthriftiness, blood around the vulva and convulsions. Signs of fetotoxicity included decreased fetal weight. Further fetal examinations were not conducted due to the severity of the maternal toxicity.

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2.12. Statistical Procedures: Statistical analyses of all data were performed as indicated below or as indicated in the individual reports from the Statistics Section:

Statistical Analyses and References

Parameter	Body Weight, Body Weight Gain, and Feed Consumption
Statistical Methods:	One-Way Analysis of Variance (ANOVA, Snedecor and Cochran, 1968) Bartlett's Test for Homogeneity of Variance (Snedecor and Cochran, 1968) and Dunnett's (Dunnett, 1964) Method of Multiple Comparisons between control and treatment groups (Dunnett, 1964). Statistically significant differences between treatment groups (e.g. low dose vs. high dose group) are not meaningful by themselves and are not discussed. The calculations derived from the ANOVA are employed in the subsequent Bartlett's and Dunnett's Tests. The primary focus of these analyses is on the results of the Dunnett's comparisons between the control and each of the treated groups. When Bartlett's Test results are highly significant (i.e., $p \leq .001$), the set of comparisons may be recalculated with the exclusion of outlier(s), (i.e., data values that are ± 2 standard deviations from the mean) or another statistical procedure may be utilized (e.g. SAS, 1983). In either case the methods are fully described.
Parameter:	Fetal Weight
Statistical Methods:	Healy Analysis (Healy, 1972).
Parameter:	Number of implantations, resorption sites, viable fetuses, % post-implantation loss, and fetal sex ratios.
Statistical Methods:	Refer to the individual Research Statistics reports located in Appendices 7.14. and 7.17.

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PC Code:	**100301**
HED File Code	**13000 Tox Reviews**
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