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

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JUL 17 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: EPA ID Number: 239-2452; Methamidophos (Monitor): Evaluation of toxicity data requested in the Registration Standard

FROM: Krystyna K. Locke, Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

Krystyna K. Locke 7/2/86

TO: W.H. Miller/ M.A. Mautz
Product Managers (16)
Registration Division (TS-767)

THRU: Edwin Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)
and
William Burnam, Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Budd 7/16/86

W.H. Burnam 7/18/86

Project No. 79
Record No. 149710

Tox. Chem. No. 378A
Accession Nos. 257622 and 257628 through 257632

Toxicology Branch/HED has completed an evaluation of the following studies with methamidophos:

17 JUL 199

	Study Type	Accession Number	Submission Volume	Core/Other Classification
1.	Oncogenic - mouse	257628	2	Guideline
2.	One-year feeding -dog	257629	3	Guideline*
3.	Pilot oral feeding (34 days) - rat	257629	3	Supplemental
4.	Chronic feeding/ Oncogenic - rat	257630 257631	4 5	Minimum* Guideline
5.	Teratogenic - rat	257632	6	Minimum
6.	Two - generation reproduction - rat	257632	6	Supplemental*
7.	Mutagenic: micronuclues test in mice	257632	6	Unacceptable
8.	Mutagenic: DNA damage in E. coli	257632	6	Unacceptable
9.	Mutagenic: reverse mutation in S. typhimurium	257632	6	Unacceptable
10.	Mutagenic: dominant lethal in mice	257632	6	Unacceptable
11.	Mutagneic: dominant lethal in mice	257632	6	Unacceptable
12.	Analysis of the test material used in above studies	257622	7	Acceptable

*These studies do not meet regulatory requirements because NOELs were not established. In studies #2 and 4 (chronic feeding), cholinesterase activity was inhibited in brain, plasma and erythrocytes at 2. 0 ppm (lowest level tested; see memorandum from Pamela M. Hurley, 6/25/86; attached). In study #6, a reproductive NOEL could not be established.

The following findings were most important:

1. Methamidophos was not oncogenic to Fischer 344 strain of rats at dose levels of 2, 6, 18 or 54 ppm and to CD-1 mice at dose levels of 1, 5 or 25 ppm, administered in the diet. Higher levels could not be used because methamidophos is a potent cholinesterase inhibitor and the animals could not survive the toxic effects resulting from this inhibition.

2. Based on cholinesterase inhibition in erythrocytes, plasma and brain, a NOEL was not established and a LOEL was 2 ppm in the two-year rat feeding study. At dose level of 2 ppm (lowest tested) cholinesterase activity was inhibited in the male and female rats as follows: in erythrocytes, 6-20%; in plasma, 7-28%; and in brain, 7-24%, when compared with the control values.

3. Based on systemic effects, a NOEL was 6 ppm and a LOEL was 18 ppm in the two-year rat feeding study. The only effect observed at the 18 ppm level was a statistically significant decrease (4-6%; $P \leq 0.05$), in body weights of male rats when related to the control values.

4. Based on cholinesterase inhibition in erythrocytes, plasma and brain, a NOEL was not established and a LOEL was 2 ppm in the one-year feeding study with beagle dogs. At dose level of 2 ppm (lowest tested), cholinesterase activity was inhibited in the male and female dogs as follow: in erythrocytes, 10-19%; in plasma, 6-23%; and in brain, 18% (M) and 11% (F), when compared with the control values.

   No systemic effects were observed at dose level of 32 ppm (highest tested).

5. Methamidophos was not teratogenic at dose level of 3 mg/kg of body weight (about 60 ppm; highest tested) to the CD strain of rats. However, at this level, signs of cholinesterase inhibition (fasciculations, hyperactivity, salivation, lacrimation and polyuria) were observed in the mothers.

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6. The two-generation reproduction study was classified as Supplemental because this study, as reported, did not provide adequate information for assessing the reproductive toxicity of methamidophos administered in diet to the CD strain of rats. Decreases in reproductive performance (percent of sperm-positive females delivering litters) were noted at all levels tested (3, 10 or 33 ppm), but nothing was reported on the sperm-positive females (4-13/dose level/generation) which did not deliver litters. The reproductive data on each animal in this study should, therefore, be submitted by the registrant. These data should include the dates at the initiation of cohabitations, dates when plugs or sperm in vaginal smears were found, uterine findings during necropsy, and findings from any method (s) used to determine the pregnancy status of females whose uteri appeared to be nonpregnant (one acceptable method is immersion of uteri in a 10 percent solution of ammonium sulfide). Since reductions in the percentage of sperm-positive females delivering litters were noted in the dosage groups in all generations in this study, the above data are essential to determine if these reductions were associated with events during fertilization, implantation, or intrauterine death after implantation.

   Since there were compound-related decrease in the number of sperm-positive females giving birth at all dose levels tested, the NOEL and, consequently, the LOEL for reproductive toxicity were not determined in this study. This study did not, therefore, satisfy the regulatory requirements.

7. The mutagenic properties of methamidophos could not be evaluated because each of the submitted studies was classified as Unacceptable by both Dynamac Corporation (contractor) and Dr. Irving Mauer, Geneticist, Toxicology Branch/HED who reviewed these studies. In general, there were deficienices in the planning, conducting and/or reporting of these studies. (Details appear in the reviews).

8. Reestablishing the ADI for methamidophos (Monitor)
   There was no ADI for Monitor for the past five years because the two IBT studies (90-day and 2-year dog feeding) on which the ADI of 0.0025 mg/kg/day was based became invalid in 1981.

The TB ADI Committee recently established a Provisional ADI (PADI) for Monitor, after considering studies summarized above. Because NOELs for the inhibition of cholinesterase activities in plasma, RBC and brain were not established in the two chronic feeding studies (1-year dog and 2-year rat) and because a NOEL (reproductive) was also not established in the 2-generation reproduction study, the Committee used the lowest level tested (3.0 ppm or 0.15 mg/kg) from the reproduction study to establish a PADI, as follows:

"TOXICOLOGY BRANCH ADI PRINTOUT Date: 06/11/86

Methamidophos	2gen reprod.- rat	PADI = 0.000150 mg/kg/day
Caswell #378A	NOEL = 0.0000 mg/kg	Safety Factor = 1000
CFR No. 180.315	LEL = 0.1500 mg/kg	

Status: TOX ADI complete 6/09/86. Pending Section Head app.* ORD not scheduLed."

*Section Head (E. R. Budd) concurred with the ADI Committee.

According to the same TOXICOLOGY BRANCH ADI PRINTOUT (06/11/86), the TMRC for published tolerances equals 0.002490 mg/kg/day (60 kg BW, 1.5 kg diet) and published tolerances represent 1660% of the PADI.

$$\frac{0.002490 \text{ mg/kg/day} \times 100}{0.000150 \text{ mg/kg/day}} = 1660\%$$

Attachments:

1. Memorandum for Pamela Hurley; 6/25/86
2. Evalaution of individual studies

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

JUL 17 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA ID Number: 239-2452. Methamidophos (Monitor^{R}). Establishment of NOEL's and LEL's for ChE Inhibition in One-Year Dog, Rat Onco/Chronic and Rat Pilot Feeding Studies.

TO: Krystyna Locke, Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 6/25/86
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Budd 7/16/86 7/18/86

Project No. 79
Record No. 149710

Tox. Chem. No. 378A
Accession Nos. 257629
through 257631

Three studies submitted by Mobay Chemical on Monitor were reviewed by Dynamac Corporation, the Contractor for the Toxicology Branch. In all three feeding studies, the NOEL for cholinesterase inhibition was considered to be 2 ppm and the LEL's were 4 ppm (pilot rat feeding), 6 ppm (chronic rat) and 8 ppm (1-year dog). After considerable discussion, including involvement of the Toxicology Branch ADI Committee, it has been decided that the NOEL's and LEL's for the three studies should be changed. It appears that in several cases, there was indeed an effect at the 2 ppm level. The following table lists the changes.

Study	ChE NOEL (tissue)	ChE LEL (tissue)
1-Year Dog	No NOEL established in any tissue.	2 ppm (brain, erythrocytes, plasma). Lowest dose tested.
Rat Chronic/ Onco	No NOEL established in any tissue.	2 ppm (brain, erythrocytes, plasma). Lowest dose tested.
Rat Pilot Feeding Study	2 ppm (erythrocytes, plasma). No NOEL established in brain.	4 ppm (erythrocytes, plasma), 2ppm (brain). Lowest dose tested.

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Questions Concerning Establishment of NOEL's for the One-Year Dog, the Two-Year Rat and the Pilot Rat Feeding Studies

Differences of opinion have occurred over the establishment of NOEL's for cholinesterase inhibition in several studies conducted on Methamidophos (Monitor). The NOEL's for this effect were originally considered to be 2 ppm in each of the three studies mentioned above. However, it is possible that there is a slight but noticeable inhibition of cholinesterase activity for brain in all three studies and for plasma and erythrocytes in the one-year dog and the 2-year rat studies. The PADI in this package is based upon 2 ppm (0.05 mg/kg/day) as the NOEL in the dog study (most sensitive species). A decision needs to be made as to whether or not 2 ppm is indeed the NOEL or if the NOEL is actually at a lower dose level. In addition to the three studies mentioned above, two subchronic feeding studies are available in which the NOEL for cholinesterase inhibition (no mention as to which tissue) is 1.5 ppm for rats (LEL 5.0 ppm) and 2 ppm (LEL 6 ppm) for dogs in erythrocytes.

N.A. Since we use the Repro-Study LEL and a 1000 fold Safety factor — However; should at a later date (i.e. after reevaluation of the new repro study) the rat study be used for an ADI determination the Committee felt that a NOEL for brain ChE was not reached in the rat. Thus the 2 ppm would be considered a LEL and a UF of 100 would be applicable

6/17/86

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EPA: 68-02-4225
DYNAMAC No. 1-45A
January 29, 1986

DATA EVALUATION RECORD

METHAMIDOPHOS

Oncogenicity Study in Mice

STUDY IDENTIFICATION: Hayes, R. H. Oncogenicity study of methamidophos technical (Monitor) on mice. (Unpublished study No. 80-332-01 prepared and submitted by Mobay Chemical Corporation, Stilwell, KS; dated August 6, 1984.) Accession No. 257628.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-29-86

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1. CHEMICAL: Methamidophos; Monitor; O,S-dimethyl phosphoramidothioate.

2. TEST MATERIAL: Methamidophos technical, batch No. 77-297-149, was described as a clear liquid containing 70 percent active ingredient.

3. STUDY/ACTION TYPE: Two-year oncogenicity study in mice.

4. STUDY IDENTIFICATION: Hayes, R. H. Oncogenicity study of methamidophos technical (Monitor) on mice. (Unpublished study No. 80-332-01 prepared and submitted by Mobay Chemical Corporation, Stilwell, KS; dated August 6, 1984.) Accession No. 257628.

5. REVIEWED BY:

Kumar D. Mainigi, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Kumar Mainigi
Date: 1-29-86

William McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 1-30-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Oncogenicity
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Signature: I Cecil Felkner
Date: 1-29-86

Krystyna K. Locke, Ph.D.
EPA Reviewer

Signature: Krystyna K. Locke
Date: 2-5-86

Edwin Budd
EPA Section Head

Signature: Edwin R Budd
Date: 5/19/86

7. CONCLUSIONS:

A. Under the conditions of the study, methamidophos was not oncogenic when fed to CD-1 mice for 106 weeks at levels of 1, 5, or 25 ppm in the diet. Malignant neoplasms, metastatic neoplasms, benign tumors, and other histopathologic lesions were found to the same extent in the controls and test animals at all levels of methami- dophos feeding. Some of these lesions were more prevalent in one sex (e.g., lymphomas in females) than the other, but were com- parable between the dietary groups. Most of the lesions reported were associated with the aging process in this strain of mice. Interstitial pneumonia observed in the 25-ppm females was also not compound related.

The systemic LOEL based on decreased gain in body weights and decreas food consumption in both sexes, is 25 ppm. A systemic NOEL en established at 5 ppm.

B. Core Classification: Guideline.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test compound, methamidophos, batch No. 77-297-149, was described as a clear liquid. The test mixture [redacted] different components. The percent of active ingredient, methamidophos, in the test mixture was determined at 6-month intervals during the study; any changes in the concentration were compensated for in preparation of the diet. The homo- geneity and stability of methamidophos in the diets were determined at regular intervals.

2. Test diets were prepared every week by homogenizing basal diet, Ralston Purina Chow 500-4 (Ettsform), with the test mixture (calculated on the basis of 70 percent active in- gredient) at 0, 1, 5, or 25 ppm. Corn oil (1 percent by weight) and acetone were used as the vehicle and solvent, respectively. Diets were stored in the freezer until fed.

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1 Only items appropriate to this OER have been included.

3. Four main groups of CD-1 mice (Charles River), 50/sex/group, were fed the diets containing 0, 1, 5, or 25 ppm of the test material for 106 weeks (termination of study). In addition, four satellite groups of 10 mice of each sex fed the same diets were maintained for laboratory investigation and terminated at week 53 of the study (interim sacrifice).

4. Animals were observed twice daily for toxic signs, moribundity, and mortality. Weekly examinations for abnormalities and masses by palpation were made. Food consumption and body weights were measured and recorded every week.

5. Mice in the satellite groups were used for hematological determinations at 6 months and 1 year. Ten randomly selected mice/sex/group were used for these determinations at the terminal sacrifice.

6. All mice found dead or sacrificed during the study or at interim and terminal sacrifices were subjected to gross necropsy. Sacrifice was by CO_2 asphyxiation. Approximately 44 tissues from each animal were fixed in formalin. Absolute and relative organ weights were tabulated after the terminal sacrifice.

7. A microscopic examination was performed on all available tissues from all animals in all groups.

8 Analysis of variance was used to assess the significance of intergroup differences, followed by least significance difference or Duncan's new multiple range test. All significant differences were reported at 95 percent confidence level.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Dietary Analysis: The chemical analysis of a composite sample of five batches of technical methamidophos revealed [redacted] (see Appendix B for identification) with an overall recovery of 98.2 to 102.4 percent. The percent active ingredient of methamidophos determined at 6, 12, 18, and 24 months ranged from 70.0 to 72.4.

Test diets fortified with [^{14}C]methamidophos at 1 ppm had an 11 percent coefficient of variation in concentration of test article between 0.2-g aliquots, indicating a homogenous distribution of the test compound.

Dietary methamidophos at 1 ppm was stable at -20°C (freezer temperature); degradation at room temperature was <15 percent for 16 days. Therefore, the test material in the diet was stable over the 7-day feeding period.

The mean, standard deviation (SD), and range of concentration for the monthly analysis (n = 25) of dietary methamidophos were as follows: 1 ppm = 0.97±0.10 (0.65-1.10); 5 ppm = 4.78±0.50 (3.89-5.90); and 25 ppm = 24.24±2.67 (20.20-30.70). A deviation of 35 percent during one analysis from the theoretical concentration occurred at 1 ppm at 24 months; deviations for 5 and 25 ppm were less than 25 percent.

B. Clinical Observations and Mortality: Ten mice found dead or sacrificed moribund during the first month of the study were replaced with new animals. These substitutes were sacrificed 1 month after termination of the study. It was reported that no dose-related effects were responsible for mortalities, although an increased death rate was seen in the 5-ppm females (Table 1).

The observed clinical signs (rough coat, urine stain, enlarged abdomen, and spontaneous seizures when handling) were reportedly related to the aging process in mice of all groups.

A total of 27 mice (14 males, 13 females) exhibited palpable masses, which were distributed as follows: abdomen, 6; neck, 5; leg, 3; perineum, 2; testicle, 2; side, 2; and rectum, back, side chin, base of tail, penis, anus, and side of leg, 1 each. These masses first started appearing in the male control group, and the average mean time of their appearance was between 50-87 weeks in males and 61-78 weeks in females. Ten mice with masses were found dead, nine were sacrificed moribund, and eight were sacrificed after 105 weeks. There was no dose-related increase in masses.

It was concluded that the test compound had no toxicological effect on behavior, occurrence of masses, or mortality.

C. Body Weight: The mean body weight of males receiving 25 ppm methamidophos was slightly (2.5-10.%) but significantly lower ($p \leq 0.05$) than controls between weeks 72 and 106, excluding weeks 75 and 87 (Table 2). Males receiving 1 ppm of the test material showed a slight (3.2%) but statistically significant decrease during week 2. Females in the 25-ppm group exhibited statistically significant decrease (5.7-13.9%) in body weights between weeks 58-106, with the exception of week 62. In addition, a statistically significant decrease (2.9-8.3%) was observed during weeks 2 and 58 for females receiving 1 ppm, and at week 26 a significant increase (3.0%) for females receiving 25 ppm.

TABLE 1. Percent Cumulative Mortality in Mice Fed Methamidophos[a]

Dietary Group (ppm)	Sex	Time (Week) 52	78	106
0	M	10 (5)	18 (9)	44 (22)
	F	10 (5)	24 (12)	44 (22)
1	M	8 (4)	18 (9)	50 (25)
	F	2 (1)	18 (9)	48 (24)
5	M	4 (2)	22 (11)	46 (23)
	F	6 (3)	32 (16)	70 (35)
25	M	4 (2)	12 (6)	54 (27)
	F	8 (4)	22 (11)	56 (28)

[a]Numbers in parentheses indicate total number of deaths that occurred beginning with day 0.

Table 2

TABLE 2. Summary of Mean Body Weight (g) in Mice Fed Different Levels of Methamidophos[a]

Methamidophos (ppm)	Week											
	60	65	70	72	75	80	85	87	90	95	100	106
						Males						
0	41	40	40	41	40	40	41	40	40	40	40	39
1	42	41	41	41	41	41	40	41	40	39	39	39
5	41	41	42	41	40	40	41	40	41	40	40	40
25	39	39	39	38*	38	38*	38*	38	39*	36*	36*	36*
	Week											
	58	60	62	65	70	75	80	85	90	95	100	106
						Females						
0	36	35	35	35	35	35	36	35	36	36	36	36
1	33*	35	34	35	34	35	35	35	34	35	35	35
5	35	35	35	35	36	35	37	36	36	35	34	36
25	33*	33*	33	33*	32*	33*	33*	32*	31*	31*	31*	32*

[a] These data were taken from Tables II (p. 27), AD03 (pp. 164-167) and AD01 (pp. 168-239) of the submission.

* Significantly different from control value ($p < 0.05$).

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D. Food Consumption: A summary of weekly food consumption at 13 weeks and 13-week intervals thereafter was given. In addition, individual weekly consumptions were reported. The consumption of methamidophos was calculated by multiplying the mean individual food consumption during the study by the dietary level of methamidophos. During week 106, all male test groups and the 5- and 25-ppm female groups consumed significantly less (males, 11.9%; females, 11.9 - 16.6%) amounts of food than controls. In addition, statistical differences between the test groups and control occurred randomly throughout the study. From week 52 until study termination, 25-ppm females consumed a significantly lesser amount of food than controls (9.1-22.0%; Table 3). Food consumption for 25-ppm males was significantly lower (4.9-13.6%) for weeks 39, 60, 78, 80, 85, 95, and 100.

E. Hematology: Hematological determinations were made at weeks 27, 54, and 105. There were no changes in hematological parameters that were consistent with time or dose. At 27 and 54 weeks, erythrocyte counts (RBC) were significantly increased in 1-ppm males; mean corpuscular hemoglobin (MCH) was increased for 5-ppm females; mean corpuscular volume (MCV) and MCH were decreased in 25-ppm males. Parameters exhibiting significant differences for different dietary levels at the same time intervals included decreased MCV for 1- and 25-ppm males at week 27, increased MCV and decreased platelets for 5- and 25-ppm males at week 54, and decreased monocytes and eosinophils for 1- and 5-ppm males at week 105. Also at week 105, there was a significant increase for RBC, hemoglobin (HGB), and hematocrit (HCT) in 25-ppm females and HCT in 25-ppm males. All of these differences were considered random and not compound-related.

F. Organ Weights: The only changes in absolute organ weights that were significant ($p \leq 0.05$) were an increase in mean brain weight in females receiving 5 ppm (3.9%) and mean lung weight in females receiving 25 ppm (23.4%). The incidence of interstitial pneumonia in the 25-ppm males was reported to be responsible for a significant increase in the absolute weights of lungs in this group. A statistically significant increase in absolute brain weight for 5-ppm females was considered to be due to the small range of absolute brain weights of control females. Organ-to-body weight ratios of adrenals, brain, heart, kidneys, and lungs showed statistically significant increases (15.4, 15.0, 20.3, 17.7, and 48.1%, respectively) in 25-ppm females (Table 4). A significant increase in the relative weights of 1-ppm female spleen (68.6%) and 25-ppm male brain (10.6%) were also recorded. The increase in relative organ weights in 25-ppm groups was considered to be due to significantly reduced body weights. Spleens in 1-ppm females were heavy because of malignant lymphomas.

G. Gross Pathology: Most commonly observed lesions in all male groups were masses within the lungs, liver, and hematopoietic tissues, especially lymph nodes, thymus, and spleen. The testes

Table 3

TABLE 3. Summary of Mean Food Consumption (g/mouse/week) in Mice Fed Various Levels of Methamidophos[a]

Methamidophos (ppm)	Week												
	39	52	60	65	70	75	78	80	85	90	95	100	106
							Males						
0	38	36	40	38	42	40	44	41	42	43	45	44	42
1	38	40*	40	40	41	41	42	41	39	42	42*	41	37*
5	36	39	39	39	41	41	42	41	40	43	42*	43	37*
25	36*	42*	37*	38	40	40	40*	39*	38*	41	40*	38*	37*
	Week												
	48	52	60	65	70	75	78	80	85	90	95	100	106
							Females						
0	38	38	42	41	45	44	44	43	45	45	50	46	42
1	36*	39	39	39	41*	41*	41	41	39*	42	47	41	37
5	40	39	41	40	43	42	44	42	41	41*	43*	41	37*
25	35*	41	37*	36*	38*	40*	39*	38*	37*	39*	39*	38*	35*

[a] This table is based on Tables I (p. 24), A004 (pp. 88-91) and A002 (pp. 92-163) of the submission.

* Significantly different from control value ($p < 0.05$).

Table 4

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TABLE 4. Mean Relative Organ Weights (% of Body Weight) of Mice Fed Different Levels of Methamidophos for 106 Weeks[b]

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Methamidophos (ppm)	Organ					
	Adrenals	Brain	Heart	Kidneys	Lung	Spleen
			Males			
0	0.052 (0.184)[a]	1.410 (0.353)	0.697 (0.195)	2.521 (1.102)	1.081 (0.816)	0.582 (0.332)[a]
1	0.025 (0.007)	1.436 (0.228)	0.716 (0.181)	2.517 (1.337)	1.255 (1.094)	0.633 (0.988)
5	0.029 (0.013)	1.431 (0.258)	0.708 (0.213)	2.521 (0.720)	1.95 (0.870)	0.568 (0.753)
25	0.038 (0.50)	1.559*(0.253)	0.779 (0.182)	2.520 (0.419)	1.248 (0.958)	0.536 (0.677)
			Females			
0	0.039 (0.010)	1.618 (0.408)	0.645 (0.207)	1.858 (0.502)	1.047 (0.376)	0.774 (0.627)
1	0.040 (0.012)	1.597 (0.266)	0.633 (0.154)	1.856 (0.291)	1.031 (0.365)	1.305*(1.684)
5	0.042 (0.013)	1.708 (0.408)	0.675 (0.170)	1.914 (0.378)	1.106 (0.336)	0.873 (0.917)
25	0.045*(0.012)	1.860*(0.331)	0.776*(0.195)	2.187*(1.052)	1.551*(0.719)	1.059 (1.255)

[a]The values in parentheses are the standard deviations.

[b]This table is based on Tables GP01 (p. 32) and GP03 (pp. 265-280) of the submission.

*Significantly different from control value ($p < 0.05$).

were often found shrunken, flaccid, and gray. Females, irrespective of their dietary group, had similar numbers of lung masses, but fewer hepatic masses and more involvement of the hematopoietic system than males. In addition, clear or red ovarian cysts and cystic uteri were often seen in females. Irrespective of sex and dietary group, animals frequently exhibited a mild to marked thickening of the glandular stomach. The kidneys were often pale, pitted, or had small cortical cysts. Intestines were often filled with fluid.

H. Histopathology: Primary malignant tumors, metastatic lymphomas, histiocytic lymphomas, myeloproliferative neoplasia, and benign tumors are tabulated in Tables 5 and 6. Dosing had no effect on the incidence or the distribution of lymphomas.

Most common primary neoplasms found in most of the dietary groups of both sexes included alveolar/bronchiolar carcinomas and hepatocellular adenomas. Malignant lymphomas were dominant in the lungs, liver, spleen, lymph nodes, kidney, and uterus, were more prevalent in females than males, but were not restricted to any dietary group. Among the benign tumors, adenomas in the lungs, Harderian, and pituitary glands were more prominent. No unusual or rare neoplasms were found. Findings were in agreement with the historical tumor data for aging albino mice.[2] Reportedly, there were no compound- or dose-related increases in total tumors, total benign tumors, animals with only single or multiple benign tumors, animals with at least one benign tumor, or animals with both benign and malignant tumors.

There was an increase in total malignant tumors in the 1- and 5-ppm females, but not in the 25-ppm females when compared to the controls. In addition, there was an increase in total malignant tumors in 5- and 25-ppm females. None of these random increases showed any dose-response relationship. It was concluded that there was no evidence for any induced oncogenicity in this study.

Nonneoplastic lesions were, in general, found at comparable incidence in control and dose groups. Common histopathologic (nonneoplastic) lesions observed in all dietary groups of both sexes included myocardial degeneration; chronic active inflammation and lymphoid hyperplasia and/or extramedullary hematopoiesis in the liver; hyperplasia of the subcapsular spindle cell and hyperplasia/alteration of the cortical glandular cell of the adrenals; hyperplasia of the glandular mucosa of the stomach; chronic interstitial inflammation, glomerular amyloidosis, and hydronephrosis of the kidneys; degeneration with occasional mineralization of the testes; and ovarian cysts and cystic endometrial hyperplasia of the uterus. Some of these lesions were more prevalent in one sex than the other, but were comparable

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[2] Percy, D. H. and Jones, A. M. "Incidence of spontaneous tumors in CD(R)-1 HaM/ICR mice." J. Natl. Cancer Inst. 46(5): 1045-1065.

Table 5

TABLE 5. Incidence of Primary Neoplastic Lesions and Malignant Lymphomas in Mice Fed Different Dietary Levels of Methamidophos[a]

Organ	Males Methamidophos (ppm) 0	1	5	25	Females Methamidophos (ppm) 0	1	5	25
Mammary Gland					50[c]	50	49	50
Adenocarcinoma	-[b]	-	-	-	1	2	3	1
Lung	50[c]	50	50	50	50	50	50	50
Alveolar/bronchiolar carcinoma	3	7	7	6	2	2	3	4
Liver	50[c]	50	50	50	50	50	50	50
Hepatocellular carcinoma	9	4	7	8	2	0	0	1
Sarcoma	0	0	0	1	2	1	0	0
Urinary Bladder	50[c]	50	50	50	50	48	50	49
Leiomyosarcoma	0	1	0	0	0	3	0	1
Pituitary Gland	50[c]	50	48	47	48	49	49	50
Carcinoma	0	0	1	1	3	4	2	0
Ovaries	-	-	-	-	50[c]	49	49	50
Sarcoma	0	0	0	0	2	0	0	0
Endometrial stromal sarcoma	0	0	0	0	1	1	2	0
Cervix	-	-	-	-	50[c]	46	45	50
Stromal cell sarcoma	0	0	0	0	2	1	1	0
Multiple Sites	50[d]	50	50	50	50	50	50	50
Lymphoma[e]	4	4	7	4	2	6	11	4
Lymphoma, histocytic	3	4	3	2	2	5	4	5
Myeloproliferative neoplasia	2	1	2	1	0	2	1	1

[a] If a neoplasm occurred at an incidence of 2 percent or less in all groups, the tumor incidence was not tabulated.

[b] (-) could not be determined from the summary table.

[c] Number of tissues examined histologically.

[d] Number of animals examined histologically.

[e] Not specified.

Table 6

TABLE 6. Incidence of Benign Tumors in Mice Fed Different Dietary Levels of Methamidophos[a]

	Males				Females			
	Methamidophos (ppm)				Methamidophos (ppm)			
n	0	1	5	25	0	1	5	25
l	50[b]	50	50	50	50	50	50	50
veolar/bronchiolar adenoma	7	5	8	7	5	10	4	8
c	50[b]	50	50	50	50	50	50	50
mangioma	0	1	1	0	0	0	2	1
nals	50[b]	50	50	50	50	50	50	50
rtical adenoma	0	1	1	0	2	1	1	0
erian Glands	50[b]	50	50	50	50	50	50	50
noma	6	4	3	2	2	4	3	4
itary Gland	-[c]	-	48	47	48	49	49	-
noma	0	0	1	1	3	4	2	0
l	-	-	-	48	-	50	-	-
toma	0	0	0	1	0	2	0	0
is	-	-	-	-	50[b]	49	49	49
nangioma	0	0	0	0	2	3	2	0
iomyoma	0	0	0	0	2	2	0	0
x	-	-	-	-	50[b]	46	45	47
iomyoma	0	0	0	0	3	3	2	2

neoplasm occurred at an incidence of 2 percent or less in all groups, the tumor incidence was not ated.

ır of tissues examined histologically.

ould not be determined from the summary table.

between the dietary groups. Nonspecific interstitial pneumonia of unknown origin was more prevalent in 25-ppm females (32/50) than 25-ppm males (15/50).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that:

1. All animals, irrespective of their dietary group, showed an age-related physical appearance and behavior. There was comparable incidence of palpable masses in all groups; however, they occurred slightly earlier in the control groups than in the test groups.

2. Food consumption and body weights for both the sexes at the 25-ppm level were significantly decreased compared to the controls.

3. There were no hemotoxic effects due to the test compound. There were no compound- or dose-related effects on organ weights or gross findings.

4. Histopathologic examinations of various tissues did not reveal any compound-induced effect on the occurrence of neoplasms. Primary neoplasms were similar in localization, type, time of occurrence, and incidence in control and test animals.

5. The NOEL is 5 ppm. The 25-ppm dose level induced toxic effects based on a significant decrease in body weights. Methamidophos was nononcogenic for male and female mice at all the dose levels tested.

B. A quality assurance statement was signed and dated August 6, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The experimental methods were complete and adequate to assess the oncogenicity of methamidophos. The report was well organized and well written. Under the conditions of the study, the test compound was clearly nononcogenic. Primary neoplasms, metastatic neoplasms, benign tumors, and other histopathologic lesions generally occurred to the same extent in the control and test animals. Two major variations were observed: 1) lymphomas were more prevalent in females and 2) interstitial pneumonia of unknown origin was found in 25-ppm females. Most of the lesions were associated with the aging process. Some of the lesions were more prevalent in one sex than the other, but were comparable between the dietary groups. Historical laboratory data on this strain of mice were available.

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There were no toxicologically important effects on the behavior, occurrence of masses, or mortality. Some relative organ weights in 25-ppm females (adrenals, brain, heart, kidneys, lungs) and 25-ppm males (brain) were significantly increased. We agree with the author's conclusion that the increase in relative organ weights was due to significant reduction in body weights. From week 60 onwards, 25-ppm females constantly consumed less amounts (7.8-22 percent) of food ($p < 0.05$) than controls. In males, food consumption started decreasing (4.8-13.6 percent) from week 78. In addition, statistical differences between the test groups and controls occurred randomly throughout the study in both sexes. Females on 25 ppm methamidophos also gained (6-19 percent) less body weight between weeks 58-106. The corresponding decrease in males was 5-10 percent less than the controls. Although it was not mentioned in the report, we assess that a systemic LOEL should be based on the decreased weight gain/food consumption at 25 ppm. We agree with the study author that the systemic NOEL is 5 ppm.

A number of inconsistencies between the text, tables, and individual data were observed:

1. Summary Tables I and II (mean feed consumption and mean body weight, respectively) contained errors. Values in the tables did not always match with the mean values given under the individual data. However, these errors were very small.

   (Appendix C includes the <u>original</u> (from the CBI) and corrected tables.)

2. <u>This submission 19</u>: The author stated "other than an increase in nonspecific interstitial pneumonia in 25 ppm males of unknown origin." In fact the summary table (CBI Vol. 2, p. 42) showed interstitial pneumonia to be a prevalent condition in females that increased with dose, 32/50 for 25-ppm females against 15/50 for 25-ppm males. Such errors reflect on the process of quality control.

Item 15--see footnote 1.

16. <u>CBI APPENDIX</u>: Appendix A, Materials and Method, CBI pp. 10-13; Appendix B, Chemical Composition of Technical Methamidophos, CBI pp. 84-86; Appendix C, Tables I and II (original and corrected), CBI pp. 24 and 27.

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APPENDIX A

Materials and Methods

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 29 through 41 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA: 68-02-4225
DYNAMAC No. 1-045-03
February 27, 1986

DATA EVALUATION RECORD

METHAMIDOPHOS

Reproductive Toxicity Study in Rats

STUDY IDENTIFICATION: Hixson, E. J. Effect of methamidophos (Monitor) on reproduction in rats. (Unpublished study No. 82-671-01 by Mobay Chemical Corp., Stilwell, KS, for Mobay Chemical Corp., Kansas City, MO, and Chevron Chemical Co., Richmond, CA; dated November 8, 1984.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-27-86

1. CHEMICAL: Methamidophos; Monitor; O,S-dimethyl phosphoramidothioate; CAS No. 10265-92-6.

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2. TEST MATERIAL: Methamidophos technical, batch No. 77-297-149, contained 70.5 percent active ingredient and was a clear liquid at room temperature; no pH was specified.

3. STUDY/ACTION TYPE: Reproductive toxicity study in rats.

4. STUDY IDENTIFICATION: Hixson, E. J. Effect of methamidophos (Monitor) on reproduction in rats. (Unpublished study No. 82-671-01 by Mobay Chemical Corp., Stilwell, KS, for Mobay Chemical Corp., Kansas City, MO, and Chevron Chemical Co., Richmond, CA; dated November 8, 1984.) Accession No. 257632.

5. REVIEWED BY:

Guillermo Millicovsky, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 26 Feb 1986

Robin B. Phipps, B.S.
Independent Reviewer
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Date: February 26, 1986

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
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Signature: Isabel Felkner
Date: 2-26-86

Krystyna K. Locke, Ph.D.
EPA Reviewer

Signature: Krystyna K. Locke
Date: 3/3/86

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/20/86

7. CONCLUSIONS:

A. We assess the NOEL and LOEL for parental toxicity at 10 and 33 ppm, respectively, based on reductions in premating and lactation body weights noted at 33 ppm. Decreases in reproductive performance were noted at 3, 10, and 33 ppm and decreases in pup viability and body weights during lactation were noted at 33 ppm. The NOEL for reproductive toxicity could not be determined for this study since compound-related effects were noted at all levels tested.

B. This study, as reported, did not provide adequate information for assessing the reproductive toxicity of the test material administered by dietary inclusion to rats. Because the NOEL could not be determined, this study did not satisfy the regulatory requirements.

Core Classification: Supplementary

8. RECOMMENDATIONS:

We recommend that reproductive data on each animal in this study be submitted. These data should include the dates at the initiation of cohabitations, dates when plugs or sperm in vaginal smears were found, uterine findings during necropsy, and findings from any method(s) used to determine the pregnancy status of females whose uteri appeared to be nonpregnant (one acceptable method is immersion of uteri in a 10 percent solution of ammonium sulfide). Since reductions in the percentage of sperm-positive females delivering litters were noted in the dosage groups in all generations in this study, the above data are essential to determine if these reductions were associated with events during fertilization, implantation, or intrauterine death after implantation. We also recommend that if additional work is conducted to assess the reproduction toxicity of methamidophos in rats, dose levels below 3 ppm should be used to determine the NOEL.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test material: Methamidophos technical, batch No. 77297-149, was described as a clear liquid at room temperature consisting of 70.5 percent active ingredient. The test material (dissolved in acetone and subsequently mixed with corn oil) was mixed with Purina Rodent Chow at concentrations of 3, 10, and 33 ppm. Control diets consisted of Purina Rodent Chow

[1] Only items appropriate to this DER have been included.

mixed with corn oil and acetone. Samples of the test diets were frozen and analyzed monthly by high pressure liquid chromatography to verify dose concentrations.

2. Test animal and test systems: A total of 104 male and 104 female CD rats were obtained from Charles River Laboratories, Kingston, NY. Animals were approximately 21 days old upon arrival to the testing laboratory. Rats were acclimated to laboratory conditions for 14 days prior to study initiation. During the course of this study, water and test diets were available ad libitum. The 26 rats/sex assigned to each group in the F_0 generation received the test or control diets for at least 100 days prior to mating. Treatments were continuous throughout the F_1 and F_2 generations. Rats were mated on a 2:1 female-to-male basis with the goal of obtaining at least 20 mated females per group. The F_1 pups were weaned at 21 days of age, and 26 pups/sex/group were randomly selected to become parents of the F_{2a} and F_{2b} generations. Pups in both F_2 generations were weaned and sacrificed at 21 days of age.

3. Parameters evaluated: Animals were examined daily for toxicologic signs. Prior to mating, animals were weighed once every week. Mated females were weighed on gestational days 0 (day of mating), 6, 13, 20, and 21; food consumption was determined for gestational days 0-7, 7-14, and 14-21. Upon delivery (lactation day 0), the number of live and dead pups, their sex, and litter weight were recorded. Maternal body weight, litter weight, number of live and dead pups, and food consumption were determined on selected days during the lactation period. Histopathologic examinations were conducted on reproductive organs and gross lesions from F_0 and F_1 parents.

12. REPORTED RESULTS:

A. Test material: The mean dose concentrations of methamidophos for the 0-, 3-, 10-, and 33-ppm levels were 0, 3.0±0.4, 9.1±1.3, and 27.9±4.0 ppm, respectively.

B. Parental effects: The most frequently reported clinical sign was alopecia. The incidence of this finding was slightly higher for the 33-ppm groups. The test material was associated with statistically significant increases (6.2%) in body weights in the premating period in F_0 male rats dosed with 10 ppm; however, males from the 33-ppm group had statistically significant body weight losses (5.4-6.7%) during the same period. Statistically significant body weight gains (5.4-13.6%) were noted in all dose groups of F_0 females prior to mating, but these were not dose related. Food consumption data from F_0 males suggested that

statistically significant increases (8.5-13.8%) and decreases (7.8-12.4%) occurred in the 10- and 33-ppm groups at various times during the premating period. Statistically significant increases (12.9-24.9%) in food consumption were reported for F_0 females from the 33-ppm group. These data are summarized in Table 1.

The group mean body weights of F_1 male and female animals in the high-dose group were significantly lower than controls at the start of the premating period and remained lower (5.9-17.1%) throughout this period. Females from the F_1 mid-dose group had statistically significant increases in body weight (7.2-12.2%) when compared with controls during the premating period. No significant differences were reported in the food consumption of F_1 males and females during this period, with the exception of the increased food intakes recorded for males (5.5-7.6%) and females (14.6-18.3%) in the 10-ppm groups. These data are summarized in Table 2.

Gestational body weight gains of F_0 rats in the 33-ppm group were significantly reduced (20.3%) when compared with controls. Body weights of F_0 animals from the other dose groups were comparable to controls. Gestational food consumption for F_0 rats from all groups was comparable. The study author reported that no biological differences were noted during lactation in F_0 maternal body weight gain or in the combined maternal and pup food consumption (Table 3). No significant effects were reported in the F_1 maternal body weight gain and food consumption during gestation or lactation of the F_{2a} generation for any group (Table 4). However, significant decreases (26.7%) were reported for the combined food consumption of F_1 mothers and their F_{2b} pups for the 33-ppm group during lactation (Table 5).

The percentage of sperm-positive F_0 females delivering F_1 pups was decreased (by 24.0-37.5%) for all dose groups compared with controls (Table 6). However, the study author did not consider this a compound-related effect due to the lack of a dose-related response in the dose groups. The percentage of F_1 females delivering F_{2a} pups was reportedly comparable for all groups; the percentage of sperm-positive F_1 females delivering F_{2b} pups was reduced (by 61.9%) for the 33-ppm group only (Table 6).

C. Offspring effects: Dose-related decreases were noted for the number of live F_1 pups at birth. The viability of pups during lactation was reduced in the high-dose group when compared with controls; however, these effects were not statistically significant. The only effect in pup viability reported for F_{2a} pups was a statistically significant decrease (23.6%) in live pups noted on day 14 of lactation for the high-dose group (Table 7). A similar (but not significant) decrease in viability was noted for F_{2b} pups from the high-dose group on day 14 of lactation.

TABLE 1. Effects of Methamidophos on Parental F_0 Rat Body Weight and Food Consumption Prior to Mating

Dose Tested	Body Weight[a] Week 1	Week 5	Week 10	Week 15	Food Consumption[a,b] Week 1	Week 5	Week 10	Week 14
Males								
Control	132 13	341 26	444 37	504 43	346[a] 16	376 29	377 22	313 23
3 ppm	133 8	333 19	437 29	491 34	296* 15	365 22	385 25	309 21
10 ppm	136 8	351 20	472* 36	535* 45	319* 19	414* 25	429* 25	350* 21
33 ppm	136 11	331 21	420* 37	470* 51	303* 18	381 22	409* 26	316 23
Females								
Control	112 6	199 16	234 21	258 22	226 9	249 16	237 18	209 14
3 ppm	114 6	206 13	251* 21	279* 29	229 15	256 22	261 33	229 38
10 ppm	115 7	205 13	248* 23	272 38	233 11	256 20	265* 29	217 39
33 ppm	113 6	203 14	258* 26	293* 37	225 10	281* 29	296* 41	248* 28

[a]Values represent group mean ± S.D., in grams.

[b]Food consumptions recorded per cage of two rats.

*Reported as "statistically different from control."

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TABLE 2. Effects of Methamidophos on Parental F_1 Rat Body Weight and Food Consumption Prior to Mating

Dose Tested	Body Weight at Week[a] 1	5	10	15	Food Consumption[a] at Week 1	5	10	15
				Males				
Control	159 29	350 37	469 48	522 61	350 23	400 30	383 35	370 32
3 ppm	146 38	335 41	455 44	508 49	339 32	389 25	382 31	364 31
10 ppm	154 28	354 34	481 41	538 52	371* 29	422* 31	410* 27	398* 30
33 ppm	134* 21	310* 30	418* 41	468* 54	350 27	397 32	371 26	356 23
				Females				
Control	129 22	209 24	252 28	271 33	268 23	253 22	241 18	240 16
3 ppm	120 25	204 21	249 24	272 32	267 17	252 15	252 17	247 13
10 ppm	133 18	224* 18	276* 23	304* 29	293 22	289 21	285* 29	275* 24
33 ppm	107* 11	187* 12	233* 16	255* 22	282 14	258 15	246 19	243 28

[a]Values represent group mean ± S.D., in grams.

*Reported as "statistically different from control."

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TABLE 3. Effects of Methamidophos on Maternal F_0 Rat Body Weight and Food Consumption during Gestation and Lactation of the F_1 Generation

Dose Tested	Body Weight[a]		Body Weight Gain[a]	Total Food Consumption[a]
	Day(s) of Gestation			
	0	21	0-21	0-21
Control	263 25	383 30	123 18	170 20
3 ppm	270 15	394 26	123 24	169 16
10 ppm	273 27	382 33	109 20	164 26
33 ppm	287* 34	385 46	98* 25	166 20
	Day(s) of Lactation			
	0	21	0-21	0-21
Control	301 34	324 25	24 25	503[b] 84
3 ppm	300 14	332 16	32 18	528[b] 101
10 ppm	305 38	327 25	24 29	512[b] 58
33 ppm	304 36	317 25	14 23	453[b] 119

[a]Values represent group mean ± S.D., in grams.

[b]Total food consumption of mother and pups.

*Reported as "significantly different from control."

TABLE 4. Effects of Methamidophos on Maternal F_1 Rat Body Weight and Food Consumption during Gestation and Lactation of the F_{2a} Generation

Dose Tested	Body Weight[a]		Body Weight Gain[a]	Total Food Consumption[a]
	Day(s) of Gestation			
	0	21	0-21	0-21
Control	278 25	379 36	101 23	145 18
3 ppm	277 21	385 25	108 22	161 34
10 ppm	311* 31	412* 40	101 27	155 24
33 ppm	259 22	343* 27	84 18	151 18
	Day(s) of Lactation			
	0	21	0-21	0-21
Control	305 26	325 24	22 13	430[b] 93
3 ppm	305 24	325 19	20 23	442[b] 101
10 ppm	333* 39	352* 28	19 26	471[b] 108
33 ppm	282* 25	289* 18	8 18	360[b] 97

[a]Values represent group mean ± S.D., in grams.

[b]Total food consumption of mother and pups.

*Reported as "significantly different from control."

TABLE 5. Effects of Methamidophos on Maternal F_1 Rat Body Weight and Food Consumption during Gestation and Lactation of the F_{2b} Generation

Dose Tested	Body Weight[a]		Body Weight Gain[a]	Total Food Consumption[a]
	Day(s) of Gestation			
	0	21	0-21	0-21
Control	311 24	428 28	115 21	153 17
3 ppm	309 19	426 25	116 19	143 19
10 ppm	342* 48	448 74	106 33	151 30
33 ppm	292 36	385 58	93 24	143 18
	Day(s) of Lactation			
	0	21	0-21	0-21
Control	336 31	331 65	-6 69	479[b] 84
3 ppm	330 17	342 22	9 27	474[b] 41
10 ppm	372 57	363 45	-9 33	410[b] 134
33 ppm	337 61	312 36	-26 31	351[b]* 108

[a]Values represent group mean ± S.D., in grams.

[b]Total food consumption of mother and pups.

*Reported as "significantly different from control."

TABLE 6. Effects of Methamidophos on Reproductive Performance of Rats

Dose Tested	No. Sperm-Positive Females	No. Litters Delivered	% Sperm-Positive Females that Delivered
	F_0 Females (Production of F_1 Generation)		
Control	25	25	100
3 ppm	23	15	65.2*
10 ppm	25	19	76.0*
33 ppm	24	15	62.5*
	F_1 Females (Production of F_{2a} Generation)		
Control	22	16	72.7
3 ppm	24	19	79.2
10 ppm	23	13	56.5
33 ppm	26	14	53.8
	F_1 Females (Production of F_{2b} Generation)		
Control	20	16	80.0
3 ppm	22	13	59.1
10 ppm	10	6	60.0
33 ppm	21	8	38.1*

* Statistically different from control value ($p \leq 0.05$); analyzed by the reviewers using Fisher's exact test.

These data are summarized in Table 7. In addition, the study author reported that the mean number of live F_{2b} pups on lactation days 0 and 14 in the high-dose group was significantly reduced when compared with controls.

Reductions in pup body weights (6.2-20.1%) during the lactation period were reported only at the 33-ppm dose level. In all generations (F_1, F_{2a}, and F_{2b}) these reductions in pup body weights were statistically significant (Table 8).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that methamidophos incorporated in the diet at a concentration of 33 ppm produced paternal, maternal, and pup body weight decreases, as well as reductions in reproductive capability in rats. No effects were noted at 10 or 3 ppm.

B. A quality assurance statement was signed and dated September 26, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Body weight data from this study were difficult to evaluate since inconsistent body weight effects were noted both within and between generations. For example, F_0 male rats dosed with 10 ppm had statistically significant body weight increases during the premating period, whereas F_0 males from the 33-ppm group had significant body weight decreases. Results also indicate that F_0 females from all three dose groups had significant body weight gains when compared with controls. However, during the same period, F_1 male and female rats dosed at 33 ppm had significant body weight decreases, whereas F_1 females dosed at 10 ppm had significant body weight increases. Maternal body weights during gestation and lactation for all generations suggested that animals in the 33-ppm groups gained less weight than controls. Maternal body weight gains for the other dose groups were not affected. Food consumption data for this study were often inconclusive and did not necessarily correlate with body weight changes. The above data are presented in Tables 1 through 5 of this review.

The reproductive performance (when measured as a function of the number of sperm-positive females that delivered pups) was adversely affected at all dose levels. We assess that the decreases in F_0 females delivering in the 3-, 10-, and 33-ppm groups were biologically and statistically significant. Moderate reductions were also noted for the number of F_1 females delivering F_{2a} litters in the 10- and 33-ppm groups. We also conclude that the percentage of animals delivering F_{2b} pups was moderately reduced for the 3- and 10-ppm groups and that a severe (and statistically significant) reduction occurred in the 33-ppm group. These data are presented in Table 6 of this review.

TABLE 7. Effects of Methamidophos on Rat Pup Viability

Dose Tested	At Birth	Day 4	Day 7	Day 14	Day 21
		Viability of F_1 Litters (%, X ± SD)			
Control	98.2 5.4	99.4 2.1	99.1 2.4	98.9 2.6	98.3 3.2
3 ppm	97.3 5.3	97.3 8.0	97.0 7.8	96.5 9.6	96.5 9.6
10 ppm	96.1 9.6	98.8 3.4	98.4 4.8	98.0 4.9	97.5 5.2
33 ppm	89.3 19.8	98.0 4.7	96.7 5.3	93.8 8.5	91.0 11.8
		Viability of F_{2a} Litters (%, X ± SD)			
Control	99.1 2.5	99.0 2.8	98.0 4.4	98.1 4.3	98.1 4.3
3 ppm	94.4 12.3	93.4 23.0	93.4 23.0	93.0 22.9	92.4 22.9
10 ppm	91.7 9.5	99.4 2.1	99.4 2.1	99.4 2.1	98.9 2.7
33 ppm	97.1 4.8	85.9 27.7	80.9 28.8	76.4* 29.2	76.4 29.2
		Viability of F_{2b} Litters (%, X ± SD)			
Control	98.3 3.6	99.4 2.5	98.9 3.1	97.7 5.5	97.7 5.5
3 ppm	100.0 0.0	98.7 3.2	97.8 3.5	97.3 4.3	96.9 5.5
10 ppm	100.0 0.0	100.0 0.0	100.0 0.0	100.0 0.0	100.0 0.0
33 ppm	95.7 6.1	84.6 35.1	81.4 33.8	74.3 33.5	73.1 34.1

*Reported as "significantly different from control."

TABLE 8. Effects of Methamidophos on Group Mean Pup Body Weight in Rats

Dose Tested	At Birth	Day 4	Day 7	Day 14	Day 21
			F_1 Pup Weight (g, X ± SD)		
Control	6.2 0.5	9.5 1.5	13.6 2.5	25.3 4.4	38.2 7.0
3 ppm	6.0 0.7	8.7 1.2	12.1 2.2	23.8 3.7	36.2 6.5
10 ppm	6.2 0.8	9.6 1.5	13.5 2.1	25.8 3.4	40.0 5.6
33 ppm	6.2 0.5	8.4* 0.9	11.2* 1.8	21.2* 3.2	32.4* 4.4
			F_{2a} Pup Weight (g, X ± SD)		
Control	6.4 0.5	9.9 1.1	13.9 1.9	26.1 3.9	39.4 6.3
3 ppm	6.3 0.4	9.4 1.3	13.4 2.0	25.0 3.5	37.6 5.7
10 ppm	6.5 0.3	10.1 1.4	14.4 2.0	26.6 4.2	40.2 6.5
33 ppm	6.0* 0.7	8.1* 1.1	11.1* 1.6	21.5* 3.4	32.1* 5.6
			F_{2b} Pup Weight (g, X ± SD)		
Control	6.4 0.5	9.9 1.2	13.6 1.9	25.1 3.7	37.9 6.4
3 ppm	6.2 0.6	8.9 1.3	11.9 1.8	22.7 3.3	32.5 4.0
10 ppm	6.5 0.6	10.1 1.9	14.0 2.9	26.8 4.8	35.5 7.5
33 ppm	6.4 0.8	9.0 1.5	11.6* 1.2	21.8 2.1	32.1 3.0

*Reported as "significantly different from control using Duncan's multiple range test."

The viability of pups during the lactation period was decreased for litters in the 33-ppm groups. The percentage of live pups on lactation day 21 was consistently reduced for the F_1, F_{2a}, and F_{2b} generations in the high-dose groups compared with controls. The viability of pups in the other dose groups appeared to be comparable to controls (Table 7).

Group mean body weights of pups during lactation were significantly reduced for 33-ppm dose groups in the F_1, F_{2a}, and F_{2b} generations. Pup body weights for the other dose groups were comparable to controls (Table 8).

8. Our conclusions differ from those of the study author with respect to the NOEL for this study. The study author assessed the NOEL at 10 ppm. However, based on compound-related decreases in the number of sperm-positive females giving birth at 3, 10, and 33 ppm we conclude that the NOEL and, consequently, the LOEL for reproductive toxicity in this study were not determined.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Methods, CBI pp. 12-15.

APPENDIX A

Materials and Methods

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 58 through 61 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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___ Description of product quality control procedures

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___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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005313

EPA: 68-02-4225
DYNAMAC No. 045-D2
January 29, 1986

DATA EVALUATION RECORD

METHAMIDOPHOS

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Hixson, E. J. Embryotoxic and teratogenic effects of methamidophos (Monitor) in rats. (Unpublished study No. 82-611-01 by Mobay Chemical Corp., Stilwell, KA, for Mobay Chemical Corp., Kansas City, MO, and Chevron Chemical Co., Richmond, CA; dated October 15, 1984.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: James R. Plaut for ICF

Date: January 29, 1986

1. CHEMICAL: Methamidophos; Monitor; o,s-dimethyl phosphoramidothioate.

2. TEST MATERIAL: Methamidophos technical (CAS Registry No. 10265-92-6), batch No. 77-297-149, was a clear liquid at room temperature and contained 70.5 percent active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Hixson, E. J. Embryotoxic and teratogenic effects of methamidophos (Monitor) in rats. (Unpublished study No. 82-611-01 by Mobay Chemical Corp., Stilwell, KA, for Mobay Chemical Corp., Kansas City, MO, and Chevron Chemical Co., Richmond, CA; dated October 15, 1984.) Accession No. 257632.

5. REVIEWED BY:

Ronald D. Hood, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: G Millicovsky FOR R.D.H.
Date: Jan 29 '86

Robin B. Phipps, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Robin B Phipps
Date: January 29, 1986

6. APPROVED BY:

Guillermo Millicovsky, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: G Millicovsky
Date: Jan 29 '86

Krystyna Locke, Ph.D.
EPA Reviewer

Signature: Krystyna R. Locke
Date: Jan. 30, 1986

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/20/86

7. CONCLUSIONS:

A. Based on the data presented, the NOEL for methamidophos technical in rats was 1 mg/kg/day for both maternal and fetal toxicity; the LOEL for both was 3.0 mg/kg/day. The maternal toxicity LOEL is based on clinical signs as well as decreased body weight gain and reduced food consumption. The LOEL for fetal toxicity is based on decreased fetal body weight. Under the test conditions employed, administration of methamidophos technical to rats was not associated with malformations; therefore, the NOEL for teratogenicity is assessed at 3.0 mg/kg/day, the highest dose administered.

B. This study is classified Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND:

A range-finding experiment conducted in 1980 (report No. 96) used groups of four pregnant rats gavaged daily with 0, 0.1, 0.3, 1.0, 3.3, or 10.0 mg methamidophos/kg/day from gestation days 6 through 20. (Confirmation of mating was designated as day 0 of gestation.) According to the study author, no adverse effects were observed at doses up to 1 mg/kg. At 3.3 and 10 mg/kg, organophosphate-related toxic effects occurred in the dams, and both maternal and fetal body weights were affected.

The study report described other preliminary work (report number not given) using dams dosed at 0.5, 1.5, or 4.5 mg/kg. Dams dosed at 4.5 mg/kg aborted their litters around day 15 of gestation. The author reported that dams dosed at 1.5 mg/kg carried their litters until sacrifice on day 21.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix A for details.)

A. The dosing mixtures consisted of technical grade methamidophos (Monitor) dissolved in distilled water. Doses were given daily by gavage at levels of 0 (vehicle control), 0.3, 1.0, or 3.0 mg/kg/day. A positive control group was dosed with hydroxyurea at 350 mg/kg in distilled water on gestation days 9, 10, and 11 only.

---

[1] Only items appropriate to this DER have been included.

B. The test animals were from Charles River Laboratories, Inc. Female CD rats were mated with males of the same strain. Mated females were dosed on gestation days 6-15 (sperm day = day 0). They were killed on day 21, C-sectioned, and the fetuses were subjected to teratological examination.

C. The parameters evaluated included:

1. Maternal
   a. clinical health--observed daily
   b. food consumption--measured through gestation days 6, 13, and 21
   c. body weights on gestation days 0, 6, 13, and 21 as well as body weight changes
   d. corpora lutea, implantations, and resorptions
   e. gravid uterine weight
   f. limited gross pathology

2. Embryonic/Fetal
   a. mortality
   b. body weight
   c. placental appearance
   d. sex
   e. gross abnormalities
   f. visceral abnormalities examined by methods described by Wilson
   g. skeletal abnormalities

12. REPORTED RESULTS:

A. Test Material Analysis. The study author stated that according to "preliminary work," stock solutions of the test material were stable for 1 week when refrigerated. Dosing solutions were made by dilution from a stock solution that was prepared weekly and stored under refrigeration. Aliquots of the stock and dosing solutions were said to have been analyzed during the range-finding study, yielding recoveries of 74.6-115 percent, with a mean of 97.3±9.5 percent. Individual analytical results and methods were not given, and no analyses were reported for the definitive study. A sample of the test material was frozen and preserved in the study archives.

B. Food Consumption and Body Weights. The relevant data are presented in Table 1. According to the study author, both the body weight and food consumption of high-dose rats (3 mg/kg/day) were significantly decreased by gestation days 13 and 21 when compared to vehicle control values. Body weight gains (total and corrected for gravid uterine weight) were also significantly reduced for high-dose females. No effects on food intake, body weight, or weight gain were seen at lower doses.

TABLE 1. Summary of Mean Maternal Food Consumption and Body Weights of Methamidophos-Treated Rats

	Dosage (mg/kg/day)			
	0 (Vehicle)	0.3	1.0	3.0
Food Consumption (g)				
Days of Pregnancy				
0- 6	146±18 (22)[a]	141±22 (25)	141±22 (26)	147±13 (27)
6-13	176±21 (22)	175±18 (25)	171±20 (26)	125±23*(27)
13-21	223±21 (22)	216±20 (24)[b]	218±24 (26)	185±25*(27)
Body Weight (g)				
Day of Pregnancy				
0	241±19 (22)	232±16 (25)	236±23 (26)	237±16 (26)
6	273±26 (22)	263±22 (25)	265±29 (26)	269±20 (26)
13	296±24 (22)	287±24 (25)	290±29 (26)	265±18*(25)[b]
21	401±28 (22)	390±30 (25)	395±37 (26)	356±30*(26)
Total Body Weight Gain (g)				
Days of Pregnancy				
0-21	160±17 (22)	158±21 (25)	160±24 (26)	119±24*(26)
Corrected Body Weight Gain (g)[c]				
Days of Pregnancy				
0-21	58±12 (22)	59±16 (25)	61±18 (26)	33±11*(26)

[a] Number in parenthesis is the sample size; numbers outside the parenthesis are the mean ± standard deviation.

[b] Data not recorded for one dam.

[c] Total body weight gain less gravid uterine weight.

*Significantly different from the control value ($p \leq 0.05$).

C. Clinical and Necropsy Observations and Mortalities. There were no significant clinical findings for the low- or mid-dose groups, but the high-dose dams exhibited signs of toxicity typical of animals exposed to a cholinesterase inhibitor. In general, these signs appeared on days 6, 7, or 8 of gestation, continued to termination, and included fasciculations, hyperactivity, salivation, lacrimation, and polyuria. Such symptoms were seen in all high-dose dams, but were not observed in controls or in rats at lower doses of methamidophos. One high-dose female displayed vaginal bleeding on day 20 of gestation, but nevertheless carried her litter to term. All females survived until the termination of the study. The findings at necropsy were unremarkable.

D. Reproductive Parameters. The appropriate data are shown in Table 2. No differences were seen among groups in terms of reproductive success. Most mated dams in all groups were found to be pregnant, with similar numbers of corpora lutea and implantations when C-sectioned. No litters were totally resorbed, aborted, or delivered prematurely.

E. Fetal Evaluation. No significant differences were seen between methamidophos-treated groups and vehicle controls with regard to litter size or prenatal mortality (Table 2). Mean fetal body weights and total litter weights of high-dose fetuses were significantly lower than vehicle control values. No compound-related increases in fetal malformations or variations were reported.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Study Author's Conclusions

1. No effects were seen in either dams or offspring at methamidophos dosages of 0.3 or 1.0 mg/kg/day.

2. At the high-dosage level (3.0 mg/kg/day), methamidophos given on gestation days 6-15 caused maternal toxicity and decreased fetal weights.

3. The NOEL of methamidophos for maternal and fetal toxicity was 1.0 mg/kg/day, whereas the NOEL for embryotoxicity and teratogenicity was 3.0 mg/kg/day.

4. The positive control, hydroxyurea, did not produce intoxication in dams dosed at 350 mg/kg. Extensive fetal effects were observed, including gross, visceral, and skeletal abnormalities.

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TABLE 2. Summary of Reproductive Parameters and Fetal Data in Rats Following Treatment with Methamidophos

	Dosage (mg/kg/day)			
	0 (Vehicle)	0.3	1.0	3.0
ernal and Litter Data:				
ams mated/pregnant	24/22	25/25	26/26	27/26
ams dying	0	0	0	0
itters aborted or delivered early	0	0	0	0
itters totally resorbed	0	0	0	0
iable litters on day 21	22	25	26	26
orpora lutea (X±SD)	16.6±2.5	15.8±2.8	16.6±2.7	17.0±3.9
mplantations (X±SD)	15.7±1.4	14.4±2.0	15.0±2.4	14.6±3.4
Resorbed (X)	6.8	3.0	5.3	5.0
ead fetuses (X)	0	0	0	0
ive fetuses (X±SD)	14.7±1.8	14.0±2.0	14.2±2.5	13.9±3.3
l Data:[a]				
ex ratio (% M/F)	48/52	51/49	52/48	51/49
ody weight (X±SD, g)	4.9±0.4	5.1±0.3	5.0±0.3	4.6±0.4*
otal litter wt. (X±SD, g)	72.7±11.3	70.9±9.6	70.6±11.7	63.0±14.6*

nificantly different from vehicle control ($p \leq 0.05$).

significant methamidophos-related increases were observed in fetal malformations, iations, or developmental delays.

B. Quality Assurance Measures. A statement was included with the study report indicating that the study had been reviewed by a QA unit in compliance with GLP regulations. A table of audit phases and audit report dates was given. The table was dated 9/26/84 and signed by R. S. Schroeder, Manager.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The reproductive health of the parental rats appeared to be good; most mated dams conceived and had normal numbers of corpora lutea and implantations. No maternal toxicity was described at the low or intermediate methamidophos dosages (0.3 or 1.0 mg/kg/day). At the high-dosage level (3.0 mg/kg/day), all of the dams exhibited signs of cholinesterase inhibitor toxicity, as described above under Section 12.C. Maternal food consumption, body weight, body weight gain, and corrected body weight gain were also significantly lower at the high-dosage level than vehicle control values. There were no maternal deaths or unusual findings at necropsy. No other measures of maternal toxicity, such as organ weights at necropsy, were available.

According to the data as summarized in Table 2, no adverse effects were seen with regard to the conceptus at either the low or the intermediate dosage. At the high dosage, the only adverse effects reported were a mild but significant decrease in mean fetal weight and a significant decrease in total litter weight. The mean total weight of the high-dosage litters was 13 percent lower than that of the vehicle control litters; this was attributable in part to a small, nonsignificant difference (0.8 fetus/litter) in the number of live fetuses per litter.

Few malformations were seen, and they did not appear to be compound related. The only possible exception would be the apparent microphthalmia (listed as "eye bulges reduced" under "gross abnormalities") seen in four fetuses in one control litter, but in four fetuses each in a different litter at the high dosage and in one fetus at the intermediate dosage. Without further information, the biological significance of such findings cannot be assessed. The distribution and types of developmental variations appeared to be unremarkable.

B. There were no differences between the study author's conclusions and those of the reviewers.

C. The study report, as presented, had the following deficiencies:

1. Although the concentrations of the test chemical were said to have been analytically determined during the preliminary pilot study, they were not monitored during the teratogenicity study.

2. Some minor discrepancies were noted in the data tabulation, but these did not alter the study interpretation.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 7-10.

APPENDIX A

Materials and Methods

Methamidophos toxicology review

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___ Information about a pending registration action

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EPA: 68-02-4225
DYNAMAC No. 045B-1
December 11, 1985

005313

DATA EVALUATION RECORD

METHAMIDOPHOS (Monitor)

One Year Feeding Study in Dogs

STUDY IDENTIFICATION: Hayes, R. H. One-year feeding study of methamidophos (Monitor) in dogs. (Unpublished study No. 81-174-01 and report No. 497/87474 prepared by Mobay Chemical Corporation, Stilwell, KS; sponsored by Mobay Chemical Corp., Kansas City, MO and by Chevron Chemical Corp., Richmond, CA; dated June 26, 1984.) Accession No. 257629.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 12-11-85

005313

1. CHEMICAL: Methamidophos (Monitor), O,S-dimethyl phosphoramidothioate. CAS No. 10265-92-6.

2. TEST MATERIAL: Technical methamidophos (Monitor) is a clear liquid consisting of 70 percent active ingredient obtained from batch No. 77-297-149. The material was stored in glass bottles, frozen (-23°C) until used, and then stored at room temperature for about 4 weeks as they were being used.

3. STUDY/ACTION TYPE: One year feeding study in dogs.

4. STUDY IDENTIFICATION: Hayes, R. H. One-year feeding study of methamido- phos (Monitor) in dogs. (Unpublished study No. 81-174-01 and report No. 497/87474 prepared by Mobay Chemical Corporation, Stilwell, KS; sponsored by Mobay Chemical Corp., Kansas City, MO and by Chevron Chemical Corp., Richmond, CA; dated June 26, 1984.) Accession No. 257629.

5. REVIEWED BY:

Paul Wennerberg, D.V.M., M.S.
Principal Reviewer
Dynamac Corporation

Signature: Ira Cecil Felkner for
Date: 12-11-85

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Dec. 11, 1985

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Effects
Technical Quality Control
Dynamac Corporation

Signature: Ira Cecil Felkner
Date: 12-11-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 1/6/86

Edwin Budd, M.S.
EPA Section Head

Signature: Edwin R. Budd
Date: 7/6/86 (but do not agree with assignment of NOEL for Chr. effects — see page 3 and cover pages of TB review).

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7. CONCLUSIONS:

A. When methamidophos was fed to male and female dogs for 1 year at dietary levels of 2, 8, or 32 ppm, there were no clinical signs of toxicity, nor effects on body weights, food consumption, hematology, clinical chemistry or urinary parameters. Organ weights were not affected, and no compound-related changes in pathology were found. Cholinesterase activity was depressed in plasma erythrocytes throughout the study, and brain cholinesterase was depressed after 1 year at dietary levels of 8 and 32 ppm. Based on the cholinesterase activity, the LOEL is 8 ppm and the NOEL is 2 ppm methamidophos. No. See cover memo for corrections. K. K. Locke 6/11/86 Budd 7/6/86

B. The study is Core Guidline.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

Materials and Methods: (See Appendix A for details.)

Diets containing the test material were prepared every 3 or 4 days. The test material was mixed with acetone as the solvent and corn oil (1% by weight) as the vehicle. The test material concentration in the diet were 0, 2, 8, and 32 ppm. The prepared diet was stored in the freezer until feeding; dogs were fed the diets for 1 year. Water was available ad libitum.

Twenty-six male and 26 female 4 month old beagle dogs were obtained from Laboratory Research Enterprises, Kalamazoo, Michigan. Dogs were individually identified and housed; they were maintained in isolation for about 3 months prior to study initiation. The dogs were assigned to groups of six/sex based on body weights.

Test material composition and percent active ingredient were determined initially and at 6 month intervals. Homogeneity and stability of test material in the diet was determined at an unspecified time. Samples of diets were analyzed monthly for test material concentration.

Clinical observations were made once or twice daily. Dogs were weighed, observed, and palpated for masses weekly. Cholinesterase levels (plasma and erythrocyte) were determined for all dogs prior to initiation, twice monthly for 3 months, then every other month and at termination of the study. Brain cholinesterase levels were measured at termination. Hematology and blood chemistry analyses and urinalysis were performed on all dogs prior to initiation, monthly

[1]Only items appropriate to this DER have been included.

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for 3 months, then every other month and prior to termination. Prior to initiation and termination, all dogs had an ophthalmological examination. All dogs had a complete gross examination, and approximately 38 tissues from all dogs were examined histologically.

12. REPORTED RESULTS:

Dietary Analysis: The active ingredient concentration of the test material was 71.4 percent at initiation and 70.2 percent at termination. Appendix B (CBI pp. 84-87) presents the composition of Technical methamidophos. Diets were prepared based on concentration of active ingredient, 70.5-71.5 percent. The homogeneity of test material in the prepared diets had an 11 percent range of deviation with a 3 percent coefficient of variation. The prepared diets were reported to be stable after 21 days in the freezer or 17 days at room temperature. No analytical data were provided for homogeneity or stability. Monthly analyses of dietary concentration of the test material gave mean values of 110, 98, and 95.6 percent of the nominal concentrations at the low-, mid-, and high-doses, respectively.

Mortalities and Clinical Observations: No mortalities occurred during the study. Clinical observations revealed lacrimation, diarrhea, loose stools, and vomiting in both sexes at all levels including controls. None of these findings were considered compound related even though the incidence was somewhat higher in mid- and high-dose dogs than in controls because there was no correspondence between the signs and inhibition of cholinesterase in individual animals.

Ophthalmologic: Ophthalmological findings were reported to be unremarkable; however, no individual animal data were provided.

Body Weights and Food Consumption: No significant changes in body weights were observed in the dosed dogs. There were no compound-related changes in food consumption.

Cholinesterase Activity: Plasma, erythrocyte, and brain cholinesterase activities of male and female dogs fed the 2-ppm diet were similar to the control values. Activity in the 8- and 32-ppm dose groups were markedly lower than control (Tables 1 and 2).

Clinical Laboratory Studies: Analyses of clinical chemistry, hematology, and urinalysis parameters did not reveal any compound-related changes. Significant changes ($p \leq 0.05$) from the control group occurred sporadically and were not consistent with time or dose.

Pathology: Gross necropsy findings did not indicate a compound- or dose-related effect. No compound-related effects were seen in mean organ weights.

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TABLE 1. Selected Average Cholinesterase Activity (μmol/ml/min) for Dogs Fed Methamidophos for 1 Year

Sex	Dietary Level (ppm)	Cholinesterase Activity[a] Baseline[b]	Week 2	Month 3	Month 9	Month 12
			Plasma Cholinesterase			
M	0	1.59	1.74	1.50	1.83	2.00
	2	1.64	1.63 (94)	1.34 (89)	1.48 (81)	1.60 (80)
	8	1.62	1.15 (66)	1.06 (71)	1.10 (60)	1.29 (65)
	32	1.59	0.60 (34)	0.78 (52)	0.84 (46)	0.87 (44)
F	0	1.49	1.54	1.34	1.73	1.66
	2	1.51	1.38 (90)	1.21 (90)	1.34 (77)	1.46 (88)
	8	1.64	1.21 (78)	1.08 (81)	1.25 (72)	1.54 (93)
	32	1.55	0.62 (40)	0.81 (61)	0.93 (53)	1.02 (61)
			Erythrocyte Cholinesterase			
M	0	1.20	1.25	1.36	1.49	1.44
	2	1.42	1.39 (112)	1.18 (87)	1.20 (81)	1.29 (90)
	8	1.24	0.83 (66)	0.57 (42)	0.46 (31)	0.55 (38)
	32	1.49	0.37 (30)	0.38 (29)	0.28 (19)	0.28 (19)
F	0	1.27	1.26	1.49	1.60	1.57
	2	1.36	1.39 (110)	1.27 (85)	1.30 (81)	1.39 (88)
	8	1.49	0.98 (78)	0.71 (48)	0.71 (44)	0.69 (44)
	32	1.40	0.33 (26)	0.40 (27)	0.25 (16)	0.26 (17)

[a] The values in parentheses are expressed as percent of control; calculations by our reviewers.

[b] Average of 3 prestudy values.

TABLE 2. Brain Cholinesterase Activity (μmol/g/min) in Dogs Fed Methamidophos for 1 Year[a]

Dietary Level (ppm)	Males	Females
0	6.17	6.01
2	5.05 (82)	5.33 (89)
8	2.79 (45)	3.28 (55)
32	1.80 (29)	2.02 (34)

[a] The values in parentheses are expressed as percent of control; calculations by our reviewers.

Histopathological evaluations did not reveal any compound-related changes. Inflammatory changes (salivary gland, lung, thyroid, and lymph nodes) were noted in dogs of both sexes from all study groups. It was stated that "trends indicating a dose or compound effect could not be established". No evidence of neoplasia in any animal was present.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The following were the conclusions of the study author:

"Methamidophos acted as a typical cholinesterase inhibitor. In summary, the following items were seen."

1. "The consumption of methamidophos at 2, 8 or 32 ppm in the diet of male or female dogs for one year did not result in differences in behavior or physical condition from the control dogs when observed daily or weekly."

2. "There were no adverse effects on feed consumption in male or female dogs."

3. "Body weights were not affected for male or female dogs consuming 2, 8 or 32 ppm methamidophos in the feed."

4. "The cholinesterase activity in plasma, erythrocyte and brain of male and female dogs consuming 2 ppm methamidophos was not affected."

5. "Hematology blood chemistry and urinalysis was not affected by consumption of methamidophos at dietary levels up to and including 32 ppm."

6. "No adverse changes were seen in the eyes of male or female dogs at any dietary level when examined ophthalmoscopically."

7. "Gross necropsy did not reveal any compound-related changes in tissues or organ weights (absolute and relative)."

8. "Abnormalities seen in organs and tissues when examined microscopically were not compound-related."

9. "The no-effect level was 2 ppm."

B. A quality assurance and G.L.P. statement were signed and dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the study author that the decrease cholinesterase activities measured for plasma, erythrocytes, and brain were the major adverse effect of feeding the test material. There were apparent dose-related trends; the slight decreases in cholinesterase activity in dogs receiving 2 ppm methamidophos are assessed to be of no toxicologic significance since they never exceeded 20 percent. The author did not analyze cholinesterase activity data statistically.

We assess that the clinical signs and inflammatory changes presented do not clearly demonstrate adverse toxicological effects of feeding the test material. However, an increase in the incidence of lacrimation was noted in dosed males and females and an increase in salivation was noted on examination of individual animal findings by our reviewers. This may have been part of a general parasympathetic response to the test material.

The study design was adequate, the summary data were supported by individual animal data, and the histopathology incidence tabulation indicated that nearly all tissues from each animal were examined histologically.

It is our assessment that the LOEL, based on cholinesterase inhibition, is 8 ppm and the NOEL 2 ppm.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A -- Materials and Methods, CBI pp. 9-15; Appendix B -- Composition of Technical Methamidophos, CBI pp. 84-86.

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 83 through 94 are not included in this copy.

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___ Identity of product inert ingredients

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EPA: 68-02-4225
DYNAMAC No. 1-45-C
December 13, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Chronic Feeding/Oncogenicity Study in Rats

STUDY IDENTIFICATION: Hayes, R. H. Chronic feeding/oncogenicity study of technical methamidophos (Monitor) to rats. (Unpublished study No. 81-271-01 by Mobay Chemical Corporation, Toxicology Department, Stillwell, KA; sponsored by Mobay Chemical Corporation Agricultural Chemicals Division, Kansas City, MO, and by Chevron Chemical Company, Agricultural Chemicals Division, Richmond, CA., dated November 13, 1984.) Accession Nos. 257630-257631.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-12-85

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7. CONCLUSIONS:

Methamidophos at dietary levels of 2, 6, 18, or 54 ppm was not oncogenic in male or female F344 rats. Body weights were decreased in males receiving 18 and 54 ppm and in females receiving 54 ppm. There was a dose-related decrease in brain, erythrocyte, and plasma cholinesterase activities at 6, 18, and 54 ppm. At the highest dose tested, brain cholinesterase activity was 75-80 percent inhibited, and plasma and erythrocyte cholinesterase activities were 75-95 percent inhibited. Based on cholinesterase activity, the LOEL is 6 ppm and the NOEL is 2 ppm. No. See cover memo for corrections K.K.Locke 6/25/86 Rdd 7/6/86

The study is considered Core Guideline for oncogenicity but Core Minimum for chronic toxicity because urinalysis data were not provided.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (Complete Materials and Methods are in Appendix A. The following is a summary.)

Fischer 344 rats obtained from Charles River Laboratories were acclimated for 10 days, and dosing was initiated when the animals were 6 weeks old. Groups of 50 rats/sex were fed diets containing 0, 2, 6, 18, or 54 ppm methamidophos (calculated as active ingredient) for 106 weeks. Satellite groups of 10 animals/sex/dose level were sacrificed at 12 months, and 10 animals/sex from the control and 2-ppm replacement rats were sacrificed at 1 month for plasma, erythrocyte, and brain cholinesterase determinations.

Diets were prepared weekly and stored frozen until presented to the animals. One percent corn oil was used as the vehicle and acetone was used as the solvent to prepare the diet. Test material was analyzed at 6-month intervals, and diets were analyzed monthly to determine concentrations of test material.

Animals were observed twice daily for toxic signs, moribundity, and mortality and received detailed examinations (including palpation) weekly. Body weights and food consumption were determined weekly. Hematologic and blood chemistry parameters were analyzed at study initiation and at 6-month intervals. Plasma and erythrocyte cholinesterase activities were determined at the same intervals as well as at 1 and 15 months. Brain cholinesterase was measured at 12 months (10 animals/sex/group) and on all animals at termination. Brain cholinesterase was also measured on 10 animals/sex (replacement animals) in control and the 2-ppm groups at 1 month.

[1] Only items appropriate to this DER have been included.

A gross necropsy was performed on all rats that died, that were sacrificed moribund, that were sacrificed at 12 months, or at study termination. Adrenals, brain, gonads, heart, kidneys, liver, lungs, and spleen were weighed. A complete set of tissues (approximately 46 organs/rat) were fixed and all available tissues were examined microscopically for all animals except those at interim sacrifice.

Statistical analysis was performed on body weight, food consumption, hematology, clinical chemistry data, and absolute and relative organ weight data using analysis of variance followed by least significant difference or Duncan's new multiple range test.

B. Protocol: The protocol was not included with the report; Materials and Methods, Appendix A, may be used for this purpose.

12. REPORTED RESULTS:

Dietary Analysis: The test material was analyzed at 6-month intervals, and percent of active ingredient was compensated for when preparing the diets. Methamidophos was homogeneously distributed in the diets. Test material was stable in feed at -20°C; at room temperature there was less than 15 percent loss over 16 days. Table 1 summarizes results of monthly analysis of diets.

TABLE 1. Dietary Analysis of Methamidophos

Nominal Level (ppm)	Mean±SD (ppm)	Range (ppm)
2	1.99±0.26	1.51± 2.50
6	5.92±0.49	5.02± 7.00
18	17.37±1.74	14.20±22.40
54	51.96±4.28	40.20±59.00

Clinical Observations and Mortality: After 20 weeks there was an increase in clinical signs such as loose stools, urine staining of fur, rough coat, and skin lesions in males and females fed dietary levels of 18 or 54 ppm.

Mortality was not markedly different in dosed and control groups; there were more deaths in dosed females than in controls but no dose-related trend in mortality. At 105 weeks, survival ranged between 74-80 percent in male groups and 64-82 percent in female groups (Table 2).

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TABLE 2. Mortality and Percent Survival in Rats Fed Methamidophos for 2 Years

	Dietary Level (ppm)				
	0	2	6	18	54
Males	13(74)[a]	12(76)	10(80)	17(66)	12(76)
Females	9(82)	17(66)	18(64)	12(76)	15(70)

[a]The value in parenthesis is percent survival.

Body Weights and Food Consumption: Table 3 summarizes mean body weight data. Mean body weights were decreased in males receiving 18 and 54 ppm and in females receiving 54 ppm. The differences were significant for males dosed 54 ppm from week 3 until termination and for males dosed 18 ppm at most intervals between weeks 5 and 84. Body weights for females receiving 54 ppm were significantly lower than controls from week 11 to study termination. Mean food consumption for all groups including controls was erratic and there were no dose-related trends.

Hematology: There were random but significant differences in hematologic parameters between dosed and control rats; however, the differences were slight, not consistent with dose or time, and not considered of toxicologic importance.

Clinical Chemistry: There were no toxicologically important changes in parameters testing liver function or kidney function when dosed groups were compared to controls. Statistically significant differences were noted, but they were neither consistent with time nor dose.

Cholinesterase: There was a dose-related decrease in plasma, erythrocyte, and brain cholinesterase in males and females when compared to controls (Tables 4 and 5). The decreases in the 2-ppm groups were not considered of toxicologic importance by the report author (see Section 14).

TABLE 3. Mean Body Weights at Intervals in Rats Fed Methamidophos for 2 Years

Sex	Dietary Level (ppm)	Mean Body Weight (g) at Week - 13	26	52	78	106
Males	0	321.3 ±19.9	359.0 ±22.7	418.2 ±28.2	430.2 ±30.2	382.4 ±26.9
	18	302.1* ±22.4	343.4* ±26.0	402.8* ±29.6	414.2* ±36.5	378.2 ±54.8
	54	286.2* ±21.4	329.6* ±20.9	365.1* ±24.3	362.6* ±27.1	334.8* ±35.2
Females	0	170.8 ±10.7	190.6 ±10.0	227.5 ±16.9	251.6 ±20.0	264.6 ±30.8
	18	170.8 ±11.1	190.1 ±10.9	227.2 ±15.6	257.8 ±27.2	266.1 ±34.8
	54	165.0* ± 9.9	179.4* ± 8.6	208.7* ±12.0	228.7* ±18.3	234.1* ±27.8

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

TABLE 4. Plasma and Erythrocyte Cholinesterase (ChE) Activities in Rats Fed Methamidophos for 2 Years

Dose Level (ppm)	Percent of Control							
	Plasma ChE at Month				Erythrocyte ChE at Month			
	6	12[b]	18	24	6	12[b]	18	24
Males								
0	100	100	100	100	100	100	100	100
	(0.55)[a]	(0.74)	(1.22)	(1.55)	(1.59)	(1.77)	(1.86)	(1.51)
2	87.3	82.4	68.5	71.6	93.7	83.6	79.6	87.4
6	80.0	62.2	58.2	52.9	64.8	58.2	58.6	68.2
18	40.0	37.8	36.1	29.7	27.0	34.5	30.6	35.1
54	20.0	16.2	12.3	9.0	21.4	21.5	18.8	24.5
Females								
0	100	100	100	100	100	100	100	100
	(2.38)[a]	(2.77)	(2.89)	(2.42)	(1.47)	(1.48)	(1.58)	(1.55)
2	79.0	73.3	93.0	89.3	87.1	87.8	89.9	81.3
6	58.8	49.5	61.6	73.6	57.1	60.8	58.2	63.9
18	26.9	37.8	36.1	29.7	27.0	34.5	30.6	35.1
54	20.0	16.2	12.3	9.0	21.4	21.5	18.8	24.5

[a]The value in parenthesis is units of activity in controls expressed as μmol/min/mL.

[b]Reserve animals, not main group, were used so that brain cholinesterase could be measured on the same animals.

TABLE 5. Mean Brain Cholinesterase Activity in Rats Fed Methamidophos for 2 Years[a]

Dose Level (ppm)	Males				Females			
	Month 12		Month 24		Month 12		Month 24	
	Units ± SD	% of Control	Units ± SD	% of Control	Units ± SD	% of Control	Units ± SD	% of Control
0	13.8±0.4	100	12.1±0.9	100	14.1±1.1	100	11.8±2.2	100
2	12.4±0.6	89.9	10.6±0.8	87.6	10.7±0.5	75.9	11.0±0.3	93.2
6	8.0±1.0	58.0	7.4±1.3	61.2	7.8±0.4	55.3	8.2±0.2	69.5
18	4.5±0.3	32.6	4.3±0.9	35.5	4.2±0.2	29.8	4.3±0.6	36.4
54	3.2±0.3	23.2	2.5±0.4	20.7	2.9±0.2	20.6	2.9±0.8	24.6

[a]Units are expressed as μmol/min/g tissue (10 animals/sex/group). Mean brain cholinesterase at 1 month was 84 percent of the control value in males receiving 2 ppm and 82 percent of control in females receiving 2 ppm. Activity at higher dose levels was not measured at 1 month.

Organ Weights: Absolute and relative brain weights were increased in males and females receiving 54 ppm. Absolute weights of liver, kidney, and testes were decreased in males receiving 18 and 54 ppm and heart decreased in males receiving 54 ppm. Weights relative to body weight that differed from control were liver (18 ppm) and testes (18 and 54 ppm) in males; kidney in females receiving 2, 6, and 54 ppm and heart in females receiving 54 ppm. Table 5 summarizes significant changes in absolute and relative organ weights. The author did not consider the changes in organ weights to be compound or dose related. The differences were within the laboratory's normal range for untreated rats. Historical data were supplied.

Gross Pathology: There were no increases in any gross lesions in dosed rats compared to controls. Incidence of gross lesions were not summarized, but were correlated with lesions found microscopically. A check of individual animal records for control and high-dose males (by the reviewers) indicated a generally good correlation between gross findings and histopathology.

Histopathology: Table 7 summarizes incidence of neoplastic lesions. There was no increase in tumors related to dosing and the neoplasms observed were common to Fischer 344 rats. The most frequently observed tumors were interstitial cell tumors of the testis, endometrial stromal polyps of the uterus, pituitary adenoma and carcinoma, adrenal pheochromocytoma, hepatocellular adenoma, adrenal cortical adenoma, islet cell tumors of the pancreas, C-cell tumors of the thyroid, and mononuclear cell leukemia of the reticuloendothelial system. The author stated that the tumors seen in this study were common to the Fischer 344 rat[2] and any increases in incidence were random and not dose related. The author reported that onset of tumors was similar between control and dosed groups.

There were no nonneoplastic lesions that were dose or compound related. The author stated that the incidence was comparable to those reported in the literature for untreated F344 rats. Frequently occurring lesions noted by the report author are summarized in Table 8. There was no apparent increase in incidence in dosed groups. These were considered age-related spontaneous lesions.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Methamidophos was not oncogenic in male or female rats fed dietary levels of 2, 6, 18, or 54 ppm. Neoplasms noted grossly and histologically were "similar in type, localization, time of onset and incidence" in control and dosed animals.

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[2] The author cited (a) NIH Rodents 1980 Catalogue, USDHHS, April 1981, Pub. No. 81-606 and (b) Representative Historical Control Data, Hazleton Laboratories America, Inc., 1984.

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TABLE 6. Mean Organ Weight and Organ-to-Body Weight Ratios in Rats Fed Methamidophos for 2 Years

Dietary Level (ppm)	Brain		Liver		Kidney		Testes		Heart	
	g	%	g	%	g	%	g	%	g	%
Males										
0	2.07	0.60	16.91	4.79	3.78	1.08	4.92	1.35	1.29	0.37
18	2.09	0.63	15.37*	4.41*	3.43*	1.01	3.45*	0.96*	1.27	0.39
54	2.12*	0.69*	14.37*	4.59	3.22*	1.04	3.06*	0.97*	1.17*	0.38
Females										
0	1.86	0.76	10.60	4.26	2.41	0.98			0.96	0.40
18	1.89*	0.75	11.39	4.47	2.60*	1.03			0.97	0.39
54	1.91*	0.91*	10.04	4.60	2.35	1.10*			0.95	0.45*

Significantly different from control value ($p < 0.05$).

TABLE 7. Primary Neoplastic Lesions in Rats Fed Methamidophos for 2 Years[a]

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Organ/Neoplasm	Males Dose Level (ppm) 0	2	6	18	54	Females Dose Level (ppm) 0	2	6	18	54
Skin/Subcutis	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Keratoacanthoma	1	0	3	1	0	2	0	3	0	2[d]
Squamous cell carcinoma	0	1	0	1	2	0	1[d]	0	1[d]	1
Fibroma	1[d]	1	2	2	1	1	0	1	0	0
Fibrosarcoma	0	1	0	0	0	0	0	0	0	0
Basal cell tumor	2	2	0	0	0	0	0	0	0	0
Basal cell carcinoma	1	0	0	0	2[d]	0	0	0	0	0
Sebaceous adenoma	1	0	0	0	0	0	0	0	0	0
Sarcoma	0	1	0	0	0	0	0	1	0	1[d]
Mammary Gland	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adenoma	0	2	0	0	0	3	2	1	2	0
Fibroadenoma	0	0	1	1	0	3	4	4	4[d]	2[d]
Liver	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma	4	7	11[c]	9	1	7	4	6	7	6
Hepatocellular carcinoma	0	0	0	1	0	0	0	0	0	0
Spleen/Multiple organs	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia	11	10	13	13	9	7	17	10	11	12
Lymphoma	0	0	0	2	0	2	0	1	0	0
Pancreas	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Islet cell adenoma	2	4	9[c]	4	3	1	0	1	0	0
Islet cell carcinoma	0	0	1	2	0	0	0	0	0	0
Adrenal	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Cortical adenoma	1	0	5	7[c]	0	2	2	4	9[c]	2
Pheochromocytoma (B)	9	6	13	2	6	0	5	2	0	2
Pheochromocytoma (M)	1	0	1	3	0	0	0	0	0	0
Testis	(50)[b]	(50)	(50)	(49)	(50)					
Interstitial cell tumors	42	45	46	41	43					
Pituitary	(49)[b]	(50)	(50)	(49)	(49)	(50)	(50)	(50)	(50)	(48)
Adenoma	27	14	27	24	21	19	25	23	29	19
Carcinoma	0	0	0	2	0	6	2	3	3	2
Uterus						(50)[b]	(50)	(50)	(50)	(49)
Endometrial stromal polyp						13	14	18	12	16
Endometrial stromal sarcoma						1	1	0	0	2
Adenoma						1	1	0	0	0
Adenocarcinoma						0	1	1	1	0

[a] If only single neoplasms were found in any group they were not tabulated.

[b] Number of tissues examined histologically.

[c] Significantly different from control incidence ($p \leq 0.05$); analysis by the reviewers using the Fisher exact test.

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TABLE 8. Frequently Occurring Nonneoplastic Lesions in Rats Fed Methamidophos for 2 Years

Organ/Lesion	Males Dose Level (ppm) 0	2	6	18	54	Females Dose Level (ppm) 0	2	6	18	54
Kidney	(50)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Chronic nephropathy	50	42	40	39	46	43	41	35	37	45
Adrenal	(50)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Medullary hyperplasia	17	23	21	18	18	11	7	15	14	9
Cortical hyperplasia	24	29	25	26	29	25	22	20	34	32
Liver	(50)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Bile duct hyperplasia	45	44	47	36	27	35	22	20	28	18
Chronic inflammation	31	29	21	17	29	23	28	27	30	27
Pancreas	(50)[a]	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)
Acinar cell atrophy	22	15	12	14	25	16	15	9	15	15
Testes	(50)[a]	(50)	(50)	(49)	(50)					
Atrophy	2	3	6	5	11					
Seminal Vesicles	(49)[a]	(50)	(50)	(49)	(50)					
Atrophy	33	35	32	33	36					
Spinal Cord	(48)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Minimal demyelination	20	25	24	17	24	30	31	32	29	27
Heart	(50)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Myocardial degeneration	23	24	24	20	23	45	42	33	38	45
Thyroid	(50)[a]	(50)	(50)	(48)	(50)	(49)	(48)	(50)	(47)	(50)
C-cell hyperplasia	10	17	18	16	14	14	10	16	9	14

[a]The number of tissues examined histologically.

Behavior and appearance in animals receiving 2 and 6 ppm were similar to controls. Most rats receiving 18 and 54 ppm had such signs as loose stool, urine stain, rough coat, and skin lesions. Body weights were decreased in males receiving 18 and 54 ppm and in females receiving 54 ppm. Cholinesterase activity in plasma, erythrocytes, and brain were not affected at 2 ppm but there was a dose-related inhibition at higher concentrations. Hematology and blood chemistry were not affected and evaluation of organ weight data did not reveal a compound or dose effect. The NOEL was determined to be 2 ppm methamidophos based on cholinesterase activity.

A quality assurance statement signed and dated November 12, 1984, was present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the conclusion of the author that methamidophos was not oncogenic when fed to rats at levels of 2-54 ppm in the diet. The incidence of tumors in general was similar to historical incidence in untreated Fischer 344 rats. There were some tumor incidences in rats receiving 6 or 18 ppm that were significantly higher ($p \leq 0.05$) than control incidences when analyzed with the Fisher exact test (see Table 7). However, in no case was there a dose-related trend, and incidence at the highest dose was comparable to control incidence. Hepatocellular adenoma was significantly increased ($p \leq 0.05$) when compared to controls in males receiving 6 ppm (22 versus 8 percent) but not in males receiving 18 or 54 ppm. National Toxicology Program (NTP) historical incidence[3] for adenomas and neoplastic nodules of the liver is 3.4±3.5 percent and Hazleton[4] historical incidence is 7.4 percent (ranging from 2-12 percent). In this study, hepatocellular adenoma probably included neoplastic nodules because the latter finding was not recorded for any animals. Pathologists often do not distinguish between neoplastic nodules and adenomas of the liver. Adrenal cortical adenomas occurred in 14 percent of males and 9 percent of females receiving 18 ppm, but the incidence in the groups receiving 54 ppm was as low or lower than the normal historical range (0 percent males, 4 percent females). Islet cell adenoma occurred in 9/50 males receiving 6 ppm, but incidence was low at higher doses. The NTP historical incidence is 3.8±3.6 percent[5] and the Hazleton historical incidence is 2.9 percent (range 0-8 percent).[6] Our assessment is that these increased incidences were random or incidental and not related to compound administration. A maximum tolerated dose was used in the study, supported by decreased weight gain in males receiving 18 and 54 ppm and females receiving 54 ppm.

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[3] Haseman, J.K., J. Huff, and G.A. Boorman. Toxicol. Path. 9(1984): 122-135.

[4] Hazleton Laboratories America, Inc. Representative Historical Control Data, 1984.

[5] Haseman et al., op. cit. pp. 122-135.

[6] Hazleton Laboratories America, Inc., op. cit.

Changes in organ weights that were significantly different from control were not indicative of a toxic effect. There were no histologic correlates, the changes were small, and when compared to historical values presented by the author (CBI pp. 55-56), the changes were not unusual. Organ weights were presented for approximately 50 animals/sex/group and included animals that died. Normally they are only given for 10 animals/sex/group or for animals that were sacrificed by design.

The report author did not present statistical analysis of cholinesterase activity data. However, there were some statistically significant differences ($p \leq 0.05$) from control in the cholinesterase activity of rats receiving 2 ppm when data were analyzed by our reviewers (ANOVA followed by Duncan's test for multiple comparisons). However, we assess that the author's interpretation that these effects were not of toxicologic importance was defensible and correct. In females receiving 2 ppm, plasma and brain cholinesterase activities were 73 and 76 percent, respectively, of control at 12 months. However, the control values were higher than expected and plasma cholinesterase activity of a second group of females at 12 months and 15 months showed that activity was 87 and 94 percent, respectively, of control values. At 24 months, plasma cholinesterase activity in males receiving 2 ppm was 72 percent of controls (Table 4) but was in the range of historical controls (1.45±0.38 U/mL).

It is our assessment that the LOEL, based on cholinesterase inhibition, is 6 ppm and that the NOEL is 2 ppm methamidophos.

The study design was adequate with the exception that urinalysis data were not presented. Gross pathology data were not tabulated in a summary but they correlated with histologic findings. Minor errors were found in the summary table of neoplastic lesions of skin and mammary gland; histologic findings for grossly observed masses of skin and mammary gland were sometimes tabulated under "masses" and in some instances tabulated twice (e.g., under mammary gland as well). Correct values determined from individual pathology data are footnoted in Table 7 of the DER. Almost all of the required tissues were presented and examined histologically. The study was adequately reported and summary data were supported by individual animal data.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 9-13.
Appendix B, Composition of Technical Methamidophos, CBI pp. 101-103.

APPENDIX A

Materials and Methods

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APPENDIX B

Composition of Technical Methamidophos

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 111 through 119 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA: 68-02-4225
DYNAMAC No. 045-B2
December 11, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Pilot Feeding Study - Rats

STUDY IDENTIFICATION: Lamb, D. W., Hayes, R. H., Abernathy, J., et al. A pilot study using technical methamidophos in rats. (Unpublished study No. 80-971-01, report No. 94, by Mobay Chemical Corporation, Stanley Research Center, Stilwell, KS; dated July 29, 1980.) Accession No. 257629.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-11-85

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1. CHEMICAL: Technical methamidophos. 005313

2. TEST MATERIAL: The test material, technical methamidophos (O,S-dimethyl phosphoramidothioate), 75 percent active ingredient, batch No. 77-297-149, was a clear liquid at room temperature. It was stored frozen at -10°F.

3. STUDY/ACTION TYPE: Pilot Feeding Study/Rats.

4. STUDY IDENTIFICATION: Lamb, D. W., Hayes, R. H., Abernathy, J., et al. A pilot study using technical methamidophos in rats. (Unpublished study No. 80-971-01, report No. 94, by Mobay Chemical Corporation, Stanley Research Center, Stilwell, KS; dated July 29, 1980.) Accession No. 257629.

5. REVIEWED BY:

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Principal Reviewer
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Date: Dec. 11, 1985

Robert J. Weir, Ph.D.
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Date: 12-11-85

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Date: 1/6/86

Edwin Budd, Ph.D.
EPA Section Head

Signature: Edwin R. Budd
Date: 7/6/86 (but believe CBE NOEL to be ≥ 2.0 ppm with marginal inhibition of brain activity at this level. See cover pages of TB review).

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7. CONCLUSIONS:

A. When methamidophos was fed to male and female Fischer 344 rats for 34 days in a pilot feeding study at levels of 1, 2, 4, 8, 16, 32, or 64 ppm, there were no deaths or toxic signs. However, there was a decreased weight gain in males receiving 64 ppm. Plasma and erythrocyte cholinesterase activities were decreased in a dose-related manner in males and females receiving dietary levels between 4 and 64 ppm. Brain cholinesterase activity was depressed at levels of 1 ppm and above. The LOEL was determined to be 4 ppm and the NOEL to be 2 ppm. There were no compound-related gross findings at necropsy.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for complete details (C8I pp. 6-8). The following is a summary.

Fischer 344 rats (Charles River), approximately 10 weeks old, were used in the study. Groups of five rats per sex were fed diets containing 0, 1, 2, 4, 8, 16, 32, or 64 ppm methamidophos for 5 weeks. Diets were stored frozen until used. Corn oil was used as a vehicle control.

Animals were observed twice daily for morbidity, mortality, or toxic and pharmacologic signs. Body weight and food consumption were monitored weekly. Plasma and erythrocyte cholinesterase levels were determined on all rats at weeks 3 and 5. Brain cholinesterase was determined at termination on all control rats and those in the groups receiving 1, 2, 4, 8, or 16 ppm.

All rats were subjected to a gross necropsy, and tissues showing gross changes were saved.

B. Protocol: Materials and Methods are submitted in lieu of protocol. See Appendix A.

12. REPORTED RESULTS:

Clinical Observations and Mortality: No toxic signs were observed in any animals. Corneal opacities were observed in one female receiving 1 ppm and one male receiving 32 ppm. There were no mortalities.

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[1]Only items appropriate to this DER have been included.

Body Weight and Food Consumption: Table 1 summarizes body weight data. The mean weight gain in males receiving 64 ppm was lower than in controls. There were wide variations in food consumption from week to week at some of the lower dose levels. There was no dose-related trend in food consumption and no correlation with weight gain or loss. The overall average food consumption (g/rat) for the 4 weeks measured are summarized in Table 2.

Gross Pathology: There were no compound-related findings at necropsy. Opacity of one eye was found in one male rat each in the 2- and 32-ppm dose groups and one female receiving 1 ppm of the test material. One male (4 ppm) had a congested thymus. Fat necrosis in the mesentery was found in a female receiving 4 ppm, and an ovarian cyst was found in a female receiving 16 ppm. Clear fluid in the uterus was found in one female from each dosed group that received 8 and 32 ppm and in three females that received 64 ppm; this finding was not considered compound related.

Cholinesterase Activity: There was a dose-related decrease in erythrocyte, plasma, and brain cholinesterase activity compared to controls at methamidophos concentrations between 4 and 64 ppm, but no appreciable depression at 1 or 2 ppm. Tables 3 and 4 summarize cholinesterase activity data.

3. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that there were no deaths and no pharmacologic or toxic signs in rats fed methamidophos for 34 days at dietary levels of 1, 2, 4, 8, 16, 32, or 64 ppm. There was a decreased weight gain at the two higher doses in males and at the highest dose in females. There were no compound-related gross findings at necropsy. There was a dose-dependent decrease in cholinesterase activity (plasma and erythrocyte) at 4, 8, 16, 32, and 64 ppm, and a marked decrease in brain cholinesterase at 4, 8, and 16 ppm in both males and females; brain cholinesterase was not measured at the two higher doses. The NOEL was considered to be 2 ppm and the MTD 32 ppm. A quality assurance statement was not included.

4. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

There was a decreased weight gain only in males receiving 64 ppm when compared to controls instead of the two highest doses, as stated by the authors (Table 1). Males receiving 1, 2, 4, 8, or 16 ppm gained more weight than controls. The initial body weights in males showed some variation because the animals were not randomized.

Our reviewers assess that the authors' other conclusions are correct and supported by the data. The study is acceptable.

TABLE 1. Mean Body Weights and Weight Gains in Rats Fed Methamidophos

Dose Level (ppm)	Males (g) Mean ± SD at Week 0	Males (g) Mean ± SD at Week 4	Males (g) Weight Gain	Females (g) Mean ± SD at Week 0	Females (g) Mean ± SD at Week 4	Females (g) Weight Gain
0	211±16.2	260±25.81	49	131± 5.8	149± 3.8	18
1	197±19.5	242±33.9	45	135± 5.8	156± 8.6	21
2	189± 7.7	243±7.1	54	131± 8.6	151± 8.6	20
4	190±22.0	248±22.4	58	136±11.7	151± 9.5	15
8	182±16.8	242±12.3	60	134±15.2	147±13.8	13
16	190±17.3	249±12.0	59	122±17.5	141±11.0	19
32	184±18.9	233±19.5	49	134± 4.2	155± 2.8	21
64	195± 8.2	225± 6.1	30	128±13.2	145± 8.7	17

TABLE 2. Mean Food Consumption in Rats Fed Methamidophos[a]

	Dose Level (ppm) 0	1	2	4	8	16	32	64
Males	458	423	421	427	436	442	437	428
Females	237	301	295	304	304	301	315	312

[a]g/rat/4 weeks.

TABLE 3. Mean Plasma and Erythrocyte Cholinesterase (ChE) Activity (as Percent of Control) in Rats Fed Methamidophos

Dose Level (ppm)	Males				Females			
	Plasma ChE at Week		Erythrocyte ChE at Week		Plasma ChE at Week		Erythrocyte ChE at Week	
	3	5	3	5	3	5	3	5
0	100	100	100	100	100	100	100	100
1	97	88	97	93	100	101	96	92
2	98	101	94	110	94	95	95	98
4	71	93	62	82	69	77	65	66
8	64	72	40	46	55	52	49	52
16	53	53	15	22	38	34	22	25
32	16	45	12	7	13	22	9	8
64	16	26	10	2	9	12	9	1

TABLE 4. Average Brain Cholinesterase Activity as Percent of Control in Rats Fed Methamidophos

	Dose Level (ppm)					
	0	1	2	4	8	16
Males	100	97	93	78	64	43
Females	100	100	88	76	58	40

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 6-8.

APPENDIX A

Materials and Methods

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 128 through 131 are not included in this copy.

The material not included contains the following type of information:

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___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

#1

005313

EPA: 68-02-4225
DYNAMAC No. 1-45-07
December 9, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Mutagenicity - Micronucleus Test for Mutagenic Potential in Mice

STUDY IDENTIFICATION: Herbold, B. SRA 5172, Methamidophos, Tamaron active ingredient micronucleus test on the mouse to evaluate for mutagenic effect. (Unpublished study No. T0000686 and report No. 9707 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany for Mobay Chemical Corporation, Kansas City, MO; dated January 22, 1981.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-9-85

005313

1. CHEMICAL: SRA 5172, Methamidophos, Tamaron, thiophosphoric acid-O,S-dimethylesteramide, ($C_2H_8NO_2PS$).

2. TEST MATERIAL: SRA 5172 (active ingredient of Tamaron); purity 62.6%.

3. STUDY/ACTION TYPE: Mutagenicity - Micronucleus Test for Mutagenic Potential in Mice.

4. STUDY IDENTIFICATION: Herbold, B. SRA 5172, Methamidophos, Tamaron active ingredient micronucleus test on the mouse to evaluate for mutagenic effect. (Unpublished study No. T0000686 and report No. 9707 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany for Mobay Chemical Corporation, Kansas City, MO; dated January 22, 1981.) Accession No. 257632.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-9-85

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 12-9-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 12-9-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 1/6/86

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/21/86

7. CONCLUSIONS:

A. Under the conditions of this study, 10 and 20 mg/kg (total dosage) of SRA 5172, administered via oral gavage to male and female mice in two single applications separated by a 24-hour interval, did not significantly increase the frequency of micronuclei in polychromatic erythrocytes collected six hours following the second administration. However, no conclusions can be drawn relative to the clastogenic potential of the test material because the sampling intervals were insufficient to assess the entire hematopoietic cycle. Also, it is not known if the chemical reached the target organ, especially since there was no sign of bone marrow depression.

B. The study was unacceptable.

8. RECOMMENDATIONS:

A. It is recommended that the assay be performed with multiple sampling intervals. In addition to 6 hours post second compound administration, at least 24 and 48 hours samples should be taken to ensure that cells are exposed to the test material during the entire hematopoietic cycle and that compound effects related to mitotic delay are adequately assessed.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

1. The test material, SRA 5172, methamidophos, was described as the insecticidal and acaricidal active ingredient of Tamaron. A refrigerated, premixed sample of the test material with a purity of 62.6 percent was used in the assay.

2. Test Animals: Eight to 12-week-old male and female mice NMRI/(WT) were obtained from F. Winkelmann, Borchen. At the initiation of the study, animals weighed 23-27 g.

3. Animal Maintenance: Animals were housed in Makrolon cages, type I with food (Altromin 1324, Altromin GmbH, Lage) and tapwater available ad libitum. The environment was controlled for temperature (23°C) relative humidity (55-66%) and light (12 hours). Animals were randomly assigned to test groups by the PH-Dokumentatien Biometrie plan.

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[1] Only items appropriate to this DER have been included.

4. Test Compound Administration: The test material was suspended in a 0.5 percent Cremophor emulsion; the positive control, Trenimon (triaziquone), was prepared in water. Test material suspensions and the negative controls were administered via oral gavage; trenimon was administered intraperitoneally. Dosing solutions were prepared to yield volumes of 10 ml/kg.

5. Micronucleus Test:

   a. Test Animals and Compound Administration. Based on the results of a preliminary study in which five animals were administered a total dosage (T.D.) of 10, 20, or 40 mg/kg in two single test material applications, two doses were selected for the micronucleus assay. Ten mice (5 males, 5 females) per dose were administered the appropriate concentration of the test material (10 or 20 mg/kg, T.D.) vehicle, or positive control, Trenimon (0.25 mg/kg, T.D.), in two single applications separated by 24 hour intervals.

   b. Animal Sacrifice/Bone Marrow Harvest. Six hours following the second compound administration, animals were sacrificed by decapitation and femoral marrow smears were prepared as described by Schmid[2].

   c. Slide Analysis. One thousand polychromatic erythrocytes (PCE) per animal were scored to establish the incidence of micronuclei; the number of normochromatic erythrocytes (NCE) per 1000 PCE was also determined.

6. Evaluation Criteria: The author stated, "A difference was considered statistically significant if the error probability was below 5 percent ($p < 0.05$)."

7. The statistical method used was the Nemenyi nonparametric rank-sum test; a reference was not provided. Data generated from the positive control group were not statistically analyzed.

B. Protocol:

Materials and Methods submitted in lieu of protocol (see Appendix A).

12. REPORTED RESULTS:

Micronucleus Assay: Based on the results of a preliminary study which were not reported, the two doses chosen for the micronucleus

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[2] W. Schmid, "The Micronucleus Test," Mutation Research 31 (1975): pp. 9-15.

assay were 10 and 20 mg/kg (T.D.) of the test material. With the exception of severe convulsions noted in one male and one female exposed to 20 mg/kg, no other toxic signs were observed in the main assay. No statistically significant increase in the frequency of micronucleated erythrocytes occurred in any test group. The ratios of PCE:NCE for animals exposed to the two doses of the test material were comparable to the vehicle control. Representative data are presented in Table 1.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded, "In closing it may be stated that no indication of a mutagenic effect for SRA 5172 in doses up to 2 x 10 mg/kg body weight per os were found in the micronucleus test on the mouse, i.e., in a somatic mutagenicity test system in vivo."

B. No quality assurance statement was present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was well conducted and the author's interpretation of the data was correct. Sampling of bone marrow cells six hours after the second compound administration (30 hours after 1st compound treatment) conforms to Schmid's rationale, which ensures that the largest proportion of cells are exposed to the test material during first and second division.[2] However, studies conducted by Salamone et al.[3] have shown that for certain compounds, the maximum frequency of PCEs with micronuclei may occur much later than 30 hours. Since PCEs have a lifespan of approximately 24 hours, the U.S. Environmental Protection Agency Gene-Tox Program has recommended sampling at 24, 48, and 72 hours after first application in a two-dose schedule to ensure that the intervals over which maximum frequencies of micronuclei are known to occur are evaluated.[4]

Target cell toxicity as indicated by an inhibition of erythropoiesis was not observed. However, the findings from the preliminary study (data not presented in which 2 x 10 mg/kg was tolerated with slight effects, and the severe convulsions seen in one male and one female following exposure to 2 x 10 mg/kg in the definitive study, suggested that this level approximated the maximum tolerated dose.

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[3] M. J. Salamone, J. A. Heddle, E. Stuart and M. Kate, "Towards an Improved Micronucleus Test: Studies on Three Model Agents, Mitomycin C, Cyclophosphamide and Dimethylbenzanthracene," Mutation Research 74 (1980): 347-356.

[4] J. A. Heddle, M. Hite, B. Kirkhart, K. Mavournin, J. T. MacGregor, G. W. Newell, and M. F. Salamone. "The Induction of Micronuclei as a Measure of Genotoxicity. A Report of the U.S. Environmental Protection Agency Gene-Tox Program," Mutation Research 123 (1983): 61-118.

005313

TABLE 1. Representative Results of the Micronucleus Assay in Mice with SRA 5172

bstance	Dose mg/kg	No. of[a] Animals Analyzed Per Group	No. of PCEs Analyzed Per Group	Total[b] No. of MPE Per Group	Percent[b] MPE Per Group	Average[b] Group PCE:NCE
hicle Control						
% Cremophor	-	10	10,000	26	0.26	1:1.1
sitive Control						
enimon	2 x 0.125	10	10,000	537	5.4	1:1.4
st Substance						
A 5172	2 x 5.0	10	10,000	21	0.21	1:1.3
	2 x 10.0[c]	10	10,000	16	0.16	1:1.2

ve male and five female per treatment.

lculated by our reviewers.

e male and one female showed toxic signs (severe convulsions).

= Polychromatic erythrocytes.
- Micronucleated polychromatic erythrocytes.
= Normochromatic erythrocytes.

The sensitivity of the assay to detect genotoxicity 30 hours following the first compound administration or six hours after the second treatment was adequately demonstrated by the marked induction of micronuclei in animals exposed to the positive control (Trenimon, 2 x 0.125 mg/kg, ip). However, sampling intervals were inadequate, especially for later intervals, to ensure that all micronuclei induced could be detected.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI pp. 4-6.

Methamidophos toxicology review

Page _____ is not included in this copy.

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___ Description of the product manufacturing process

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___ A draft product label

___ The product confidential statement of formula

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X FIFRA registration data

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005313

#8

EPA: 68-02-4225
TASK: 45-08
December 4, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Mutagenicity--DNA Damage in Escherichia coli

STUDY IDENTIFICATION: Herbold, B. Pol test on E. coli to evaluate for DNA damage with SRA 5172. (Unpublished study No. 12318/86395 and report No. 12318 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation, Kansas City, MO; dated December 19, 1983.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-4-85

005313

1. CHEMICAL: SRA 5172; Methamidophos; O,S-dimethyl-phosphoramidothinate.

2. TEST MATERIAL: SRA 5172, batch No. 808319101, is an insecticide and acaricide and is the active ingredient in Tamaron; the test material had a purity of 71.2 percent.

3. STUDY/ACTION TYPE: Mutagenicity--DNA damage in Escherichia coli.

4. STUDY IDENTIFICATION: Herbold, B. Pol test on E. coli to evaluate for DNA damage with SRA 5172. (Unpublished study No. 12318/86395 and report No. 12318 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation, Kansas City, MO; dated December 19, 1983.) Accession No. 257632.

5. REVIEWED BY:

Brenda Worthy, M.T.
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Dynamac Corporation

Signature: Brenda Worthy
Date: December 4, 1985

Nancy McCarroll, B.S.
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Date: December 4, 1985

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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EPA Reviewer

Signature: Pamela Hurley
Date: 1/6/86

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/21/86

7. CONCLUSIONS:

A. Under the conditions of the assay, five dose levels ranging from 625 to 10000 µg/disc of SRA 5172 did not induce DNA damage in E. coli strains p 3478 or W 3110 with or without S9 activation. However, the inability of the test material to induce a cytotoxic response (preferential or equivalent) in the E. coli strains and the questionable growth phase of the cell cultures preclude the evaluation of this study and renders the assay invalid.

B. The study is unacceptable.

8. RECOMMENDATIONS:

A. Unless a cytotoxic response can be demonstrated in this test system (the test material had been assayed at the required maximum 10 mg/disc), the assessment of SRA 5172 to induce DNA damage should be evaluated in a mammalian cell assay, such as unscheduled DNA synthesis in primary rat hepatocytes or in WI-38 diploid human fibroblasts.

B. Cell cultures should be incubated overnight to reach an exponential growth phase.

C. The assay was performed with 30% S9. It is recommended that the assay also be performed with a lower amount (2 or 4%) of S9.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix A for details.)

A. Materials and Methods:

1. The test material, SRA 5172, batch No 808319101, was described as an insecticidal- and acaricidal-active ingredient with a purity of 71.2 percent. The test material was solubilized in dimethylsulfoxide (DMSO), the solvent control.

2. The E. coli strains used were the repair-proficient (polA+) strain W 3110 and the repair-deficient (polA-) strain p 3478. The strain source was not specified. Bacterial cultures were maintained as frozen stocks at -80°C. Prior to use, 0.1 mL of each strain was thawed, transferred to 5 mL of nutrient broth, and incubated for 30 minutes at 37°C.

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[1] Only items appropriate to this DER have been included.

3. The S9 fraction was prepared from the livers of six adult male Sprague-Dawley rats induced intraperitoneally with 500 mg/kg of Arochlor 1254. The S9 mix was prepared fresh according to Ames et al.,[2] with the S9 fraction comprising 30 percent of the S9 mix.

4. Controls: The negative (antibiotic) control used was chloramphenicol and the positive control used was methylmethanesulphonate (MMS).

5. The DNA damage assay was conducted according to the method of Rosenkranz et al.[3] in replicate plates (4) with and without S9 activation. Filter discs, impregnated with the test material, solvent, or negative or positive controls, were placed onto nutrient broth (agar) plates. The plates were incubated for 24 hours at 37°C, and the inhibition zones were measured.

6. Evaluation Criteria: The extent of DNA damage was measured by comparing the zones of inhibition of the repair-deficient and the repair-proficient strains. A test chemical was considered positive if there was a reproducible increase of more than +2 mm in diameter between the two strains.

12. REPORTED RESULTS:

SRA 5172 was assayed at dose levels of 625, 1250, 2500, 5000, and 10000 µg/plate with and without S9 activation. None of the five doses produced inhibition zones in either strain in the presence or absence of S9 activation (see Table 1 for representative results).

The positive control, MMS (10 µL, -S9 and +S9), caused a greater than 2-mm increase in the zone of inhibition around strain p 3478 (polA-) when compared to strain W 3110 (polA+), indicating the system's sensitivity.

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[2]Ames, B.N., W.E. Durston, E. Yamasaki, and F.D. Lee, "Carcinogens are mutagens: A simple test combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. (USA) 70 (1973):2281.

[3]Rosenkranz, H.S. and Z. Leifer. "Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient Escherichia coli, in: Chemical Mutagens, Principles and Methods for Their Detection, E. J. de Serres and A. Hollaender, editors (New York and London: Plenum Press, 1980) 6:109.

TABLE 1. Representative Results of DNA Damage in E. coli with SRA 5172

Substance	Dose/ disc	30% S9 Acti- vation	Zone of Inhibition[a] (mm) Repair- Proficient W 3110	Repair- Deficient p 3478
Solvent Control				
DMSO		-	0	0
Negative Control				
Chloramphenicol	30 µg	-	29.5	23.2
Positive Control				
Methylmethanesulphonate	10 µL	-	45.5	58.2
		+	43.9	59.8
Test Material[b]				
SRA 5172	625 µg[c]	-	0	0
		+	0	0
	10000 µg[d]	-	0	0
		+	0	0

[a]Averaged from four plates.

[b]The remaining dose levels (1250, 2500, and 5000 µg/disc) were comparable to the solvent control.

[c]Lowest dose tested.

[d]Highest dose tested.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: 005313

A. The author concluded that the test material assayed at doses between 625 and 10000 µg/plate "did not reveal any biologically relevant differences from the corresponding solvent control which could be interpreted as a potential for DNA damage for SRA 5172."

B. A quality assurance statement was not present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study as performed cannot be evaluated for the following reasons:

1. The test material at the highest dose tested (10000 µg/disc) was not cytotoxic for either repair-deficient or repair-proficient strains. Because inhibition (preferential or equivalent) is the only end point measured in the E. coli polA assay, the inability of the test material to induce a zone of inhibition in either strain results in a "no test."[4]

2. The use of MMS (a direct-acting mutagen) as the S9-activated positive control is inappropriate. To demonstrate the activity of the S9 mix, a promutagen that requires activation to induce genotoxic effect is mandatory.

3. The performance of the assay with cultures incubated for 30 minutes after thawing did not provide sufficient time for the cells to reach an exponential growth phase. Exponential growth phase cultures provide the optimal condition for the DNA damage assay; therefore, the sensitivity of the cell population was equivocal.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 5-8.

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[4]Rosenkranz, H.S., J. Hyman, and Z. Leifer; "DNA polymerase deficient assay," in: Evaluation of Short-Term Tests for Carcinogens, F.J. de Serres and John Asby, editors (Elsevier/North-Holland, 1981) 1:210-218.

005313

APPENDIX A

Materials and Methods

Methamidophos toxicology review

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Pages 149 through 152 are not included in this copy.

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X FIFRA registration data

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#1

005313

EPA: 68-02-4225
TASK: 45-D4
December 4, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Mutagenicity--Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Herbold, B. Salmonella/microsome test to evaluate for point mutation with SRA 5172 Tamaron active ingredient methamidophos. (Unpublished study No. 9175/69371 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Norbay Chemical Corporation, Kansas City, MO; dated May 20, 1980.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-4-85

005313

1. CHEMICAL: SRA 5172; methamidophos; Tamaron; thiophosphoric acid O,S-dimethylesteramide.

2. TEST MATERIAL: SRA 5172 is an insecticide and acaricide and is the active ingredient in Tamaron; its purity was 62.6 percent.

3. STUDY/ACTION TYPE: Mutagenicity--reverse mutation in Salmonella.

4. STUDY IDENTIFICATION: Herbold, B. Salmonella/microsome test to evaluate for point mutation with SRA 5172 Tamaron active ingredient methamidophos. (Unpublished study No. 9175/69371 prepared by Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation, Kansas City, MO; dated May 20, 1980.) Accession No. 257632.

5. REVIEWED BY:

Brenda Worthy, M.T.
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Signature: Brenda Worthy
Date: 12-4-85

Nancy McCarroll, B.S.
Independent Reviewer
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Date: 12-4-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Date: 12-4-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 4/6/86

Edwin Budd
EPA Section Head

Signature: Edwin E. Budd
Date: 5/21/86

7. CONCLUSIONS:

A. Under the conditions of the assay, 20 to 12,500 µg/plate SRA 5172 (methamidophos) did not induce a mutagenic response in S. typhimurium strains TA1535, TA1537, TA98, or TA100 with or without 30 percent S9 activation. However, the test material should also be assayed with an S9 concentration of 4 percent.

B. The study is unacceptable.

8. RECOMMENDATION:

1. To fully evaluate the potential mutagenic effect of the test material, a standard amount (4 percent) of S9 should be used in the S9 mix.

2. Although the positive controls Endoxan and trypaflavine will induce mutagenic responses in the tester strains without S9 activation, the responses are inconsistent; therefore, it is recommended that the author use known direct-acting mutagens in future studies.

3. Individual plate counts on the means and standard deviations of all test doses, solvent, and positive controls should be presented.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material, SRA 5172, was described as the active ingredient in Tamaron with a stated purity of 62.6 percent. The test material was solubilized in dimethylsulfoxide (DMSO), the solvent control.

2. The S. typhimurium strains used were TA1535, TA1537, TA100, and TA98; the source of the strains was not reported. Prior to use, 0.1 mL of each strain was thawed and transferred to 5 mL of nutrient broth at 37°C.

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[1]Only items appropriate to this DER have been included.

3. The S9 fraction was prepared from the combined livers of six adult male Sprague-Dawley rats induced by ip injection with 500 mg/kg of Aroclor 1254. The S9 mix was prepared fresh according to Ames et al.[2] with the S9 fraction comprising 30 percent of the S9 mix.

4. The positive controls used were Endoxan and trypaflavine, which are both promutagens.

5. Cytotoxicity was assessed by the reduction in the total bacterial count. The cultures used were obtained from 24-hour bacterial suspensions diluted 10^{-6} in nutrient broth and incubated at 37°C.

6. The mutagenicity assay was conducted according to the method of Ames et al.[3,4] in duplicate plates with and without S9 activation.

7. A test material was considered positive if, in at least one strain, there was a reproducible, dose-related, and twofold increase in the mutant count over the solvent control.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay: The cytotoxicity assay was conducted in parallel with the mutational assay using 20, 100, 500, 2,500, and 12,500 µg/plate of the test material without S9 activation. Cytotoxicity was observed as 75 and 41 percent reductions in viable cells for strain TA1535 and 49 and 32 percent reduction in strain TA1537 at the two

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[2]Ames, B.N., F.D. Lee, and W.E. Durston, "An improved bacterial test system for the detection and classification of mutagens and carcinogens," Proc. Natl. Acad. Sci. (USA) 70(1973b): 782-786.

[3]Ames, B.N., W. E. Durston, D. Yamasaki, and F. D. Lee, "Carcinogens are mutagens: a simple test combining liver homogenates for activation and bacteria for detection, Proc. Natl. Acad. Sci. (USA) 70(1973): 2281-2285.

[4]Ames, B.N. J. McCann, and D. Yamasaki, "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res. 31(1975): 347-364.

highest dose levels (12,500 and 2,500 µg/plate, respectively). No cytotoxicity was observed for strains TA98 and TA100. The total bacterial counts for the remaining dose levels were comparable to the solvent control. Representative results, using only the highest cytotoxic and noncytotoxic doses, are presented in Table 1.

Mutational Assay: The test material induced a twofold increase in revertants over the solvent control in strain TA1535 at the 100 µg/plate dose without S9 activation. Twofold or greater increases in his^+ revertants in strain TA1537 were seen at 100, 500, and 2,500 µg/mL without S9 activation and at 2,500 µg/mL with S9 activation (Table 2). No appreciable increase in reversion to histidine prototrophy was reported for strains TA98 and TA100 at any dose tested in the absence or presence of S9 activation. When the assay was repeated, the positive responses observed for strains TA1535 and TA1537 were not confirmed; therefore, the increases were considered to be a random fluctuation of the two tester strains (Table 3).

The positive controls Endoxan and trypaflavine caused a greater than twofold increase in revertants over the solvent control, "thus demonstrating the system's sensitivity and the activity of the S9 mix, since they require a high degree of metabolic activation to produce a mutagenic effect."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that "no indications of mutagenic effects for SRA 5172 were found in the Salmonella/microsome test in evaluable doses up to 12500 µg per plate with the test strains used."

B. A quality assurance statement was not present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the author interpreted the data correctly, and that SRA 5172 (methamidophos) did not induce a mutagenic response at doses of 20, 100, 500, 2,500, or 12,500 µg/plate in S. typhimurium strains TA1535, TA1537, TA98, or TA100 with or without S9 activation.

There was a twofold or greater increase in revertants over the solvent control with strain TA1535 at a dose of 100 µg/plate and with strain TA1537 at 100, 500, and 2,500 µg/plate nonactivated; however, on repeat analysis with strain TA1535 (-S9) at doses of 50, 100, and 200 µg/plate, the revertant response was comparable to the solvent control. Likewise, with strain TA1537 at dose levels of 100, 500, and 2,500 µg/plate, the revertant response was similar to the solvent controls with and without S9 activation.

TABLE 1. Representative Cytotoxicity Results with SRA 5172 in S. typhimurium

Substance	Dose (µg/plate)	Total bacterial count/mL (x 10^8) TA1535	TA100	TA1537	TA98
Solvent control					
DMSO		80.9	24.4	122.5	68.2
Positive controls					
Endoxan	435	79.9	21.3		
Trypaflavine	200			141.2	76.8
Test material					
SRA 5172	500[a]	53.5	22.2	109.1	53.3
	12,500[b]	20.5[c]	22.2	62.2[c]	82.5

[a]Highest noncytotoxic dose tested.
[b]Highest cytotoxic dose tested.
[c]Cytotoxicity.

TABLE 2. Results of the S. typhimurium Mutagenicity Assay with SRA 5172

Substance	Dose (µg/plate)	30% S9 Activation	Average revertants/plate[a] TA1535	TA100	TA1537	TA98
Solvent control						
DMSO		-	10.5	109.5	4.5	47.0
		+	15.3	119.8	8.0	66.8
Positive controls						
Endoxan	435[b]	-	28.0[c]	150.5		
		+	86.5[c]	370.0[c]		
Trypaflavine	200	-			92.3[c]	9.0[d]
		+			307.5[c]	1004.8[c]
Test material						
SRA 5172	20	-	16.0	115.0	4.0	41.0
	100	-	23.3[c]	108.3	10.5[c]	45.5
	500	-	15.0	118.0	14.8[c]	40.5
	2,500	-	18.0	135.0	9.5[c]	44.5
	12,500	-	17.0	136.8	6.3	40.5
	20	+	15.0	143.0	13.0	64.5
	100	+	12.5	145.5	9.7	64.5
	500	+	14.8	138.3	11.8	62.0
	2,500	+	18.8	122.5	21.0[c]	55.5
	12,500	+	16.0	156.0	12.0	55.5

[a] Averaged from duplicate plates.
[b] Equivalent to 300 µg of cyclophosphamide.
[c] Twofold or greater increase over the solvent control.
[d] Cytotoxicity.

TABLE 3. Results of Repeat S. typhimurium Mutagenicity Assay with SRA 5172

Substance	Dose (µg/plate)	30% S9 activation	Average revertants/plate[a] TA1535	TA1537
Solvent control				
DMSO		-	12.0	9.8
		+	9.8	9.5
Positive controls				
Endoxan	435[b]	-	16.5	
		+	123.0[c]	
Trypaflavine	200	-		163.0[c]
		+		193.5[c]
Test material				
SRA 5172	50	-	5.3	
	100	-	7.0	6.3
	200	-	8.0	
	500	-		6.5
	2,500	-		7.3
	100	+		7.5
	500	+		11.8
	2,500	+		12.0

[a]Averaged from duplicate plates.
[b]Equivalent to 300 µg of cyclophosphamide.
[c]Greater than a twofold increase over the solvent control.

Cytotoxicity was observed at the two highest doses, indicating that the selected dose range was adequate.

The number of revertants for the solvent control with each tester strain was within published ranges.[5]

In the S9-activated assay the positive controls Endoxan and trypaflavine responded appropriately. The revertant responses were inconsistent in the nonactivated assay; however, the positive controls did increase the number of revertants over the solvent controls, demonstrating the sensitivity of the assay to detect mutagenicity.

The positive controls used are classified as promutagens requiring S9 activation for optimum response. They will induce mutagenic responses without S9 activation in these four tester strains; however, we suggest that the author consider using a known direct-acting mutagen in future studies.

We had some concern regarding the screening of a test material with 30 percent of the S9 fraction; the recommended standard S9 mix should contain 4 percent of the S9 fraction.[6] However, there were structural similarities (Figure 1) between the test material and the positive control Endoxan (cyclophosphamide), which responds optimally with high S9 levels in the S9 mix. Therefore, we assessed that the high percentage of S9 was appropriate; however, the assay should have been conducted with both high and low levels of S9 to eliminate the question of the high S9 masking a mutagenic response.

Structure

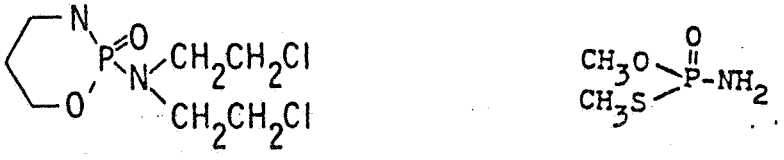

Figure 1. Chemical Structures of SRA 5172 and Endoxan (cyclophosphamide)

[5]deSerres F.J., and M.D. Shelby, "Recommendations on data production and analysis using Salmonella/microsome mutagenicity assay," Mutat. Res. 64(1979): 159-165.

[6]Maron D.M., and B.N. Ames, "Revised methods for the Salmonella mutagenicity test," Mutat. Res. 113(1983): 173-215.

Data were presented as the average of duplicate plates; it is recommended that individual plate counts or an indication of variability (i.e., standard deviations) be reported.[7]

Item 15--see footnote 1, p. 3.

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp. 5-7.

[7] deSerres, F.J., and M.D. Shelby, pp. 159-165.

APPENDIX A

(Materials, Methods, and Protocol)

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 164 through 166 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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___ Description of the product manufacturing process

___ Description of product quality control procedures

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___ Sales or other commercial/financial information

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___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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005313

EPA: 68-02-4225
DYNAMAC No. 1-45-D6
December 12, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Mutagenicity--Dominant Lethal Assay in Mice

STUDY IDENTIFICATION: Eisenlord, G. H., Carver, J. H., and Wong, Z. A. Dominant lethal study of methamidophos technical in mice. (Unpublished study No. SOCAL 1783 prepared by Chevron Environmental Health Center, Inc., Richmond, CA, for Mobay Chemical Corporation, Kansas City, MO; dated March 23, 1984.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-12-85

1. CHEMICAL: Methamidophos; Monitor. 005313

2. TEST MATERIAL: Methamidophos, technical (SX-1244, reference No. 77-297-149), had a purity of 74.3 percent.

3. STUDY/ACTION TYPE: Mutagenicity--dominant lethal assay in mice.

4. STUDY IDENTIFICATION: Eisenlord, G. H., Carver, J. H., and Wong, Z. A. Dominant lethal study of methamidophos technical in mice. (Unpublished study No. SOCAL 1783 prepared by Chevron Environmental Health Center, Inc., Richmond, CA, for Mobay Chemical Corporation, Kansas City, MO; dated March 23, 1984.) Accession No. 257632.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-11-85

Brenda Worthy, M.T.
Independent Reviewer
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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Signature: I. Cecil Felkner
Date: 12-12-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 1/6/86

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/21/86

7. CONCLUSIONS:

A. Under the conditions of this study, feeding male mice 5, 50, or 150 ppm methamidophos technical (SX-1244) for 5 days did not cause any compound-related dominant lethal effects in offspring born after an 8-week mating cycle. We conclude, however, that the route of administration was inappropriate and the levels of the test material used in this study provide no evidence that the chemical reached the gonads. Additionally, the number of mated females at each mating interval was insufficient to fully assess the mutagenic potential of the test material in the assay test system.

B. The study was unacceptable.

8. RECOMMENDATIONS:

It is recommended that the test material be evaluated in a repeat dominant lethal assay by an appropriate route that will provide evidence of transport to the gonads. A minimum of 30 fertilized females per group per mating interval should be used.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, methamidophos technical (SX-1244, reference No. 77-297-149), contained 74.3 percent methamidophos (active ingredient).

2. Test Animals: 60-day-old male and female CD-1 (ICR-derived) mice were obtained from Charles River Laboratories, Portage, MI. Throughout the course of the study, females were received at weekly intervals to ensure that no age difference between males and females at the initiation of the study or between females at each mating period existed. All animals were acclimated to laboratory conditions for 12 days.

3. Animal Maintenance/Randomization: Male mice were individually housed in polycarbonate cages; prior to mating, females were gang caged (eight to nine per cage) in polycarbonate cages. After mating, females were housed in groups of two. All animals were maintained in an air-conditioned room, controlled

[1]Only items appropriate to this DER have been included.

for temperature (73 ± 2°F) and relative humidity (50-70 percent). Throughout the course of the study, food (Purina Certified Rodent Chow #5002) and water were provided ad libitum. Animals were uniquely identified by cage cards and ear punch.

Successful breeder males were weighed 1 day prior to dosing and randomly assigned to test groups on the basis of weight (>30.5 and <39.9 g) using the Taussky-Todd overflow procedure. A reference for this procedure was not provided.

4. Test Compound Preparation: A diet containing 150 ppm of the test material was prepared by transferring 600 mg of the test material, which had been mixed in 67 mL of acetone and 40 g of corn oil, to a separatory funnel positioned over a mixing bowl containing pre-weighed, powdered rodent chow. Contents of the separatory funnel were slowly dripped onto the continuously mixed feed. An additional 133 mL of acetone was used to rinse the funnel and was introduced into the feed as described. This 150-ppm stock diet was mixed with the appropriate amounts of feed to achieve the 5- and 50-ppm diets. The control diet was prepared in a similar manner; the test material was excluded. Immediately following diet preparation, six samples were frozen for later analysis. The rationale for dose selection was not provided in this report. However, results of a pilot study indicated that methamidophos was a cholinesterase inhibitor and that the maximum tolerated dose in rats following a 34-day feeding period was 32 ppm.

5. Compound Administration: Twelve individually caged male mice per group were provided the appropriate pre-weighed diet or the control ad libitum for 5 days. At the end of the feeding interval, the mice and the remaining feed were weighed. The mean concentration of the test material in the feed following exposure was determined for each treatment group.

   Males in the positive control group were injected on day 5 with a 0.03 mg/mL aqueous solution of triethylenemelamine.

6. Dominant Lethal Assay:

   a. Mating: Individual males were caged with two virgin females 1 day after the diet was stopped or 1 day after the positive control was injected. At the end of 1 week, the females were removed and replaced with two virgin females. The weekly mating sequences were repeated for a total of 8 weeks.

   b. Animal Observations: Males were observed twice daily for mortality, overt signs of toxicity, and abnormal behavior; weekly body weights were recorded.

c. Examination and Scoring of Uteri: 8 days after each mating interval, the females were coded, sacrificed by cervical dislocation, and the uterine contents were examined. The number of implantations (live fetuses, early fetal deaths, and late fetal deaths) was determined. The uteri and implants were preserved in 10 percent buffered formalin.

d. Necropsy of Males: At the conclusion of the 8-week breeding cycle, males were sacrificed by cervical dislocation and gross pathological findings were recorded.

e. Data Evaluation: Body weights and food consumption were statistically analyzed using Student's t-test. The pregnancy index and indexes for females with one or more dead implants and for females with two or more dead implants were analyzed by Fisher's Exact Test. Implantation, fetal resorption, early death, nonviable (early and late), and the mutation (early and late deaths) indexes were analyzed by ANOVA, the Kruskal-Wallis Test, and the Wilcoxon Two-Sample Test.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Animal Observations: No toxic signs were observed in male mice fed diets containing 5, 50, and 150 ppm of the test material for 5 days. Mean body weights and food consumption for mice fed the high-dose diet of the test material were significantly lower than the controls. Food consumption for the mice in the 50-ppm group was slightly lower but not significantly different than the controls. No significant weight differences occurred in the 5- or 50-ppm test groups.

B. Diet Analysis and Intake of the Test Material: Diet analyses performed pre-dosing and at the conclusion of the 5-day feeding period indicated that the test material concentrations in the feed were 87.6, 90.6, and 95.1 percent of the target levels for the 5-, 50-, and 150-ppm diets, respectively.

C. Dominant Lethal Assay: The sequential mating of the treated male mice to virgin females did not result in significant differences in dominant lethal fetal mutations compared to the control group matings. Although a significant increase in early fetal deaths was observed at the sixth mating week for the high-dose animals, the authors concluded that this was an isolated event. The authors stated, "it is highly unlikely and atypical for a positive dominant lethal agent to induce a selective mutagenic effect at week 6 only, given the nature of the spermatogenic cycle of the mouse. In addition, the incidence of early deaths in the negative control group was extremely low in week 6. Also, no trends were observed in either week 5 or 7." Representative data from selected mating intervals are presented in Table 1.

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TABLE 1. Representative Results from Selected Mating Weeks of the Dominant Lethal Assay in Mice Exposed to Methamidophos

bstanc.		Fertility Index[a]	Total Implants	Live Implants	Early Implants Loss	Late Implants Loss	Mutation Index (early deaths)[b]	Mutation Index (late deaths)[c]
ntrol Diet		96	277	264	13	0	0.04	0.04
M[d]		96	239**	130**	106**	3	0.45**	0.46**
thamidophos	5 ppm[e]	100	282	270	11	1	0.04	0.04
	50 ppm	96	274	265	9	0	0.03	0.03
	150 ppm	96	267	261	5	1	0.02	0.02
ntrol Diet		92	272	255	15	2	0.06	0.07
M		83	233	209	22	2	0.13*	0.13*
thamidophos	5 ppm	83	247	240	7	0	0.02	0.02
	50 ppm	96	279	266	13	0	0.04	0.04
	150 ppm	100	296	280	16	0	0.05	0.05
ntrol Diet		100	311	303	8	0	0.02	0.02
M		83	245	214*	16*	2	0.06*	0.07*
thamidophos	5 ppm	92	282	271	10	1	0.04	0.04
	50 ppm	100	303	283	12	8	0.04	0.07
	150 ppm	100	302	282	20*	0	0.08*	0.08*
ntrol Diet		96	284	267	15	2	0.06	0.06
M		83	250	233	13	4	0.05	0.06
thamidophos	5 ppm	92	262	251	9	2	0.03	0.04
	50 ppm	96	261	246	15	0	0.07	0.07
	150 ppm	82[f]	202	190	12	0	0.05	0.05

Index = $\frac{\text{No. of Pregnant Females}}{\text{No. of Mated Females (24)}} \times 100$

Index (early deaths) is the average number of early deaths/total implantations; calculated by averaging in- male indexes.

Index (early and late deaths) is the average number of total deaths/total implantations; calculated by individual male indexes.

lethylenemelamine, 0.03 mg/mL.

e test material in feed (5-day feeding).

les died and the uterine contents could not be counted; they were, therefore, not included in the total

antly different from controls ($p \leq 0.05$).

antly different from controls ($p \leq 0.01$).

ues for the test material during mating weeks 2, 3, 4, and 8 were comparable to controls and were, there- e, not presented in this table.

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13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "no dominant lethal effects due to methamidophos treatment at any of the levels tested could be inferred from the data."

B. A quality assurance statement was present, signed, and dated May 30, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was well conducted and that the authors interpreted their data correctly. We agree with the authors that the significant increase in early fetal deaths observed for the 150-ppm group at the sixth mating interval was an isolated event and was probably due to the low incidence of early deaths in the negative control group. The significant increase in early fetal deaths observed only at the sixth mating week corresponded to either early meiotic (i.e., spermatocytes) or mitotic (i.e., spermatogonia) germ stages. Because these stages in the spermatogenic cycle span several weeks, positive findings should have also occurred at other mating intervals (weeks 5 or 7) for the effect to have biological relevance.

However we assess that the study could not be fully evaluated for the following reasons:

1. The route of administration was inappropriate. The test animals should have been exposed to the test material by one of the preferred routes (gavage or intraperitoneal injection)[2]

2. Because of the route of administration and the lack of a toxic or cytotoxic effect, no evidence was provided to assure that the test material was transported to the target cell (gonads).

3. The number of fertilized females at each mating interval (24) was low. The minimum number recommended for the detection of a weak mutagen is 30 fertilized females per group per mating period.[3]

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[2] Green, S., Auletta, A., Fabricant, J., Kapp, R., Manandhar, M., Shea, C., Springer, J., and Whitfield, B. Current status of bioassays in genetic toxicology--the dominant lethal assay, Mutat. Res. 154(1985): pp. 46-67.

[3] Bateman, A. J. The dominant lethal assay in the male mice in Handbook of Mutagenicity Test Procedures. B. J. Kilbey, M. Legator, W. Nichols, and C. Ramel (eds.) Elsevier, Amsterdam, NY (1977): pp. 325-335.

The statistically significant increases in nonviable implants (early and late) following a single injection of triethylenemelamine (0.03 mg/mL), which occurred at mating weeks 1, 2, 3, 5, and 6, adequately demonstrated the sensitivity of the test system to detect dominant lethality by an appropriate route.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp 2-7, 38-40.

APPENDIX A

(Materials, Methods, and Protocol)

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 176 through 184 are not included in this copy.

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EPA: 68-02-4225
DYNAMAC No. 1-45-D5
December 12, 1985

DATA EVALUATION RECORD

METHAMIDOPHOS

Mutagenicity--Dominant Lethal Assay in Mice

STUDY IDENTIFICATION: Herbold, B. Dominant lethal test on male mice to evaluate SRA 5172 for mutagenic potential. (Unpublished study Nos. 69360, SRA 5172/002 and Report No. 9583 prepared by Bayer AG Institut für Toxikologie, Wuppertal, West Germany, for Mobay Chemical Corporation, Kansas City, MO; dated November 26, 1980.) Accession No. 257632.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 12-12-85

005313

1. CHEMICAL: SRA 5172; methamidophos; Tamaron; thiophosphoric acid-O,S-dimethylesteramide ($C_2H_8NO_2PS$).

2. TEST MATERIAL: SRA 5172 (active ingredient of Tamaron) had a purity of 62.6 percent.

3. STUDY/ACTION TYPE: Mutagenicity--dominant lethal assay in mice.

4. STUDY IDENTIFICATION: Herbold, B. Dominant lethal test on male mice to evaluate SRA 5172 for mutagenic potential. (Unpublished study Nos. 69360, SRA 5172/002 and Report No. 9583 prepared by Bayer AG Institut für Toxikologie, Wuppertal, West Germany, for Mobay Chemical Corporation, Kansas City, MO; dated November 26, 1980.) Accession No. 257632.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-12-85

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 12-12-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 12-12-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 1/6/86

Edwin Budd
EPA Section Head

Signature: Edwin R. Budd
Date: 5/21/86

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7. CONCLUSIONS:

A. No meaningful conclusions could be drawn from the dominant lethal assay of SRA 5172 (methamidophos) in male mice for the following reasons:

1. The data were insufficient to show that the single selected dose (5 mg/kg) represented the maximum tolerated dose.

2. The study should have been performed with at least two and preferably three dose levels.

3. Fertility of both dosed and control groups at all mating intervals was markedly reduced and may have been related to environmental conditions, the solvent or the mouse strain.

4. Decreases in total implantation and corpora lutea and increases in preimplantation and postimplantation losses in the first five mating intervals occurred erratically; no statistically significant pattern of dominant lethality emerged.

5. The erratic results in relevant dominant lethal parameters may have been related to environmental conditions, solvent, or mouse strain.

6. A positive control group to assess the responsiveness of the test system for detecting a dominant lethal effect was not included.

B. The study is unacceptable.

8. RECOMMENDATIONS:

The following are recommendations for upgrading a repeat study:

a. Justify the selection of the high dose used or assay the maximum tolerated dose and/or a dose that is cytotoxic for the target cell (gonads), a mid dose and a low dose.

b. Select a strain of mouse and/or a vehicle control so that a fertility rate of 80 percent or greater is achieved in the control groups.

c. Include a positive control group to establish the sensitivity of the test system for detecting a dominant lethal effect.

Items 9 and 10--see footnote 1.

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[1] Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material, SRA 5172, was described as the insecticidal- and acaricidal-active ingredient of Tamaron. The assay was conducted with a refrigerated presolution sample of the test material that was 62.6 percent pure.

2. Test Animals: Eight- to 10-week-old male and female mice, strain NMRI/ORIG, Kisslegg, were obtained from S. Ivanovas GmbH, Kisslegg/Allgau. At the start of the study males weighed 30-40 g and females weighed 28-33 g.

3. Animal Maintenance/Randomization: The animals were housed in Type I Makrolon cages; the environment was controlled for temperature (24±2°C), relative humidity (44±4 percent), and light (12 hours). During mating, individual males were caged with individual virgin females; after mating females were housed separately. Throughout the study, food (Altromin 1324 chow) and tapwater were available ad libitum.

Males were randomly assigned to the test groups according to the randomization plan of Abteilung PH-Dokumentatien Biometrie.

4. Test Compound Administration: Based on the results of a preliminary study in which groups of five female mice were orally dosed with 7.5 and 15 mg/kg of the test material, the dose selected for the dominant lethal assay was 5 mg/kg. The author stated that, "7.5 mg/kg was tolerated with induction of mild symptoms, only." Fifty males per group received a single oral dose of the test material (5 mg/kg) the only dose tested, prepared in a 0.5 percent Cremophor emulsion, or the vehicle control.

5. Dominant Lethal Assay:

a. Mating: Individually treated males were caged with one untreated female immediately following treatment. Preliminary fertility studies were not conducted with the males. At the end of the 4-day mating interval, the female was removed and replaced with a new virgin female. The 4-day mating sequence was continued for a total of 48 days.

b. Observation of Females/Scoring Progeny: Females were not examined for the occurrence of vaginal plugs. Fourteen days after each mating interval, the uterus of each female was examined for pre and postimplantation losses. The fertility index was calculated, and the total numbers of live and dead implants and corpora lutea were counted. The method used to determine corpora lutea was not reported.

c. Data Evaluation: The number of dead and total implants and the ratio of dead to total implants were analyzed by analysis of variance. When appropriate, Dunnett's or Tukey's test were performed. The frequency of dead implants, viable implants, total implants, and preimplantations in the treated and control groups were compared by the nonparametric Kolmogorov-Smirnov test. A reference for the Kolmogorov-Smirnov test was not provided.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Dominant Lethal Assay: Representative data from mating intervals 1 through 6 are presented in Table 1. Representative results from all mating intervals, presented as the ratio of the test material findings for a given parameter relative to the control group, are shown in Table 2. No toxicity was observed in male mice exposed to a single oral dose (5 mg/kg) of the test material. Reduced fertility index (FI) was, however, reported for both test and control groups at all mating intervals (Table 1). The most pronounced reduction in fertility occurred at the first mating interval immediately following test material or vehicle control administration; the FI for both groups was 44. While the FI for control groups at all mating intervals was lower than the limiting fertilization rate recommended for this assay,[2] a 20 percent or greater reduction in test animal FI was calculated at mating intervals 3, 4, and 6 (Table 2).

Similarly, a 20 percent or greater reduction in the test groups' total implants and corpora lutea was observed at mating intervals 2, 3, 4, 5, and 7. Increases in preimplantation losses for test groups occurred at 1, 2, 5, 7, and 12 mating periods. At the fifth mating interval, the index of preimplantation loss/total implants in test animals when compared to control animals was 15.6. This effect was, however, not significant. Dead implant indexes of 2.0, 2.8, and 2.3 were calculated for test group matings 1, 3, and 5, respectively. Statistically significant differences in total implants (fifth mating) and in dead implants (third mating) were reported. The author concluded, however, that these significant increases were not biologically relevant. From the overall results, the author concluded that the test material was not mutagenic.

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[2] Ehling, U. H., L. Machemer, W. Buselmaier, J. Dycka, H. Frohberg, J. Kratochvilova, R. Lang, D. Lorke, D. Müller, J. Peh, G. Röhrborn, R. Roll, M. Schulze-Schencking, and H. Wiemann, "Standard protocol for the dominant lethal test on male mice. Set up by the work group 'Dominant Lethal Mutations' of the ad hoc Committee Chemogenetics," Archives of Toxicology 39(1978): 173-185.

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TABLE 1

TABLE I. Representative Results from Selected Mating Intervals of the Dominant Lethal Assay of SRA 5172 in Mice

val	Substance	Fertility Index[a]	Total Implants	Corpora Lutea/ Total Implants	Pre-implant Loss/ Total Implants	Dead Implants/ Total Implants
	Control (0.5% Cremophor)	44.0	256	260	4	7
	Test Material (SRA 5172, 5 mg/kg)	44.0	236	245	9	13
	Control (0.5% Cremophor)	57.1[b]	303	310	7	17
	Test Material (SRA 5172, 5 mg/kg)	49.0[b]	236	261	25	17
	Control (0.5% Cremophor)	58.0	305	316	11	12
	Test Material (SRA 5172, 5 mg/kg)	44.0	243	256	13	27
	Control (0.5% Cremophor)	68.0	350	380	30	22
	Test Material (SRA 5172, 5 mg/kg)	48.0	257	264	7	16
	Control (0.5% Cremophor)	62.0	343	345	2	12
	Test Material (SRA 5172, 5 mg/kg)	60.0	289	316	27	23
	Control (0.5% Cremophor)	70.0	364	376	12	18
	Test Material (SRA 5172, 5 mg/kg)	58.0	318	327	9	17

ty Index = $\frac{\text{No. of Pregnant Females}}{\text{No. of Mated Females (50)}} \times 100$

ine females mated, no explanation was given for this discrepancy.

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TABLE 2. Summarized Results of the Dominant Lethal Assay of SRA 5172 in Mice: Ratio of Individual Parameters

Mating Interval	Fertility[a] Index	Total[a] Implants	Corpora[a] Lutea	Pre-implantation Loss Index[b]	Post-implantation Loss Index[b]
1	1	0.9	0.9	2.4	2.0
2	0.9	0.8	0.8	4.2	1.2
3	0.8	0.8	0.8	1.3	2.8*
4	0.7	0.7	0.7	0.3	1.0
5	1.0	0.8**	0.9	15.6	2.3
6	0.8	0.9	0.9	0.9	1.0
7	0.9	0.8	0.9	3.3	1.3
8	0.9	1.0	1.0	1.0	1.5
9	1.1	1.1	1.1	1.5	0.6
10	0.9	0.9	0.9	1.1	1.4
11	1.0	1.0	1.0	0.6	1.5
12	1.1	1.1	1.2	4.5	0.7

[a] $\frac{\text{Findings of a given parameter (test group)}}{\text{Findings of a given parameter (control group)}}$; calculated by the reviewers.

[b] $\frac{\text{Implantation loss (pre or post)/total implants (test group)}}{\text{Implantation loss (pre or post)/total implants (control group)}}$; calculated by the reviewers.

*Significantly different from control value at $p < 0.05$, using analysis of variance.

**Significantly different from control value at $p < 0.05$, using Kolmogorov Smirnov test.

The reduced fertility, decreases in total implantation and corpora lutea, and increases in preimplantation loss and dead implants observed in the first five test-group mating intervals occurred in an erratic manner; therefore, no statistically significant pattern of dominant lethality emerged at a single mating interval or cluster of mating intervals. We also reanalyzed the data (corpora lutea, total implantations, and preimplantation and postimplantation losses) for significance. The statistical approach we employed was a two-factor nested analysis of variance with male mice nested within the dose group and crossed with the mating week.

The analysis was performed both with and without transformed data (the transformation was $y = \sqrt{x} + \sqrt{x+1}$ where x was the absolute number of dead implants per female per male). Our results confirmed the study author's findings that indicated that the single administered dose of the test material did not have a significant effect on these parameters at any mating interval. Additionally, the response did not change across the mating intervals nor was a dose-mating-period interaction observed.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI pp. 4-6.

APPENDIX A

Materials and Methods

APPENDIX A

Materials and Methods

Methamidophos toxicology review

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