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007891

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

MAY 4 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No.: 0034. Technical Methamidophos (Monitor): Additional Data Submitted by the Registrants (Chevron Chemical Company/Valent USA and Mobay Chemical Corporation) to Clarify or Upgrade Previously Evaluated Studies

FROM: Krystyna L. Locke, Toxicologist Krystyna L. Locke 4/2/90
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

TO: Richard King, PM Team No. 74
Special Review and Reregistration Division (H7508C)

THRU: Roger Gardner, Acting Section Head
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Record No.: 253395

Tox. Chem. No.: 378A
TB Project No.: 9-2293

In 1985-86, Toxicology Branch has evaluated several studies with technical methamidophos which were requested in the Registration Standard. Studies listed below either required additional information for the purpose of upgrading or did not have NOELs, or were classified as Unacceptable.

Study/Lab/Study No./ Date and Accession No./ MRID No.	Classification of Study
1. Chronic oral (1-year) - dog; Mobay Corporation; No. 81-174-01 and 497/87474; June 26, 1984. 257629/00147938	Guideline*
2. Chronic feeding/Oncogenic - rat; Mobay Corporation; No. 81-271-01 and 554/88687; November 13, 1984. 257630/00148452	Minimum*

1951

3.	Two-generation reproduction - rat; Mobay Chemical Corporation; No. 82-671-01 and 553/88686; November 8, 1984. 257632/00148455	Supplementary*
4.	Mutagenicity - Micronucleus test in mice; Bayer, West Germany; No. T0000[illegible] and 9[illegible]7; January 2[illegible], 1981. 257632/00148459	Unacceptable
5.	Mutagenicity: DNA damage in E. coli; Bayer, West Germany; No. 12318/86395; December 19, 1983. 275632/00148460	Unacceptable
6.	Mutagenicity: Reverse mutation in S. typhimurium; Bayer, West Germany; No. 9175/69371; May 20, 1980. 257632/00148456	Unacceptable
7.	Mutagenicity: Dominant lethal in mice; Chevron Environmental Health Center, Inc.; No. SOCAL 1783; March 23, 1984. 257632/00148458	Unacceptable
8.	Mutagenicity: Dominant lethal in mice; Bayer, West Germany; No. 5172/002 and 9583; November 26, 1980. 275632/0014857	Unacceptable

*These studies did not meet regulatory requirements because NOELs were not established. In studies #1 and #2 (chronic feeding), cholinesterase activity was inhibited in brain, plasma and erythrocytes at 2.0 ppm (lowest level tested). In study #3, a reproductive NOEL could not be established from the reported data and additional data were requested.

The current submission contains a) additional data for the 2-generation rat reproduction study; b) supplemental (not requested) data for the following studies: rat chronic feeding/oncogenicity, mouse oncogenicity and 1-year dog feeding; and c) 2 new mutagenic studies (unscheduled DNA synthesis in rat hepatocytes and cytogenetics study in mice). Based on these additional data, the rat reproduction study has been upgraded to Core-Minimum and the systemic/reproductive/developmental NOEL and LEL have now been established at 10 ppm (0.5 mg/kg) and 33 ppm (1.65 mg/kg), respectively. The classifications of the 3 long-term feeding studies (rat, dog and mouse) remain unchanged and

cholinesterase NOELs are still not established in the rat and dog studies. Both mutagenicity studies are acceptable; technical methamidophos was not mutagenic in these studies. (See Attachments I-V for details.)

Attachment I

007891

One-Year Feeding Study of Methamidophos (MONITOR^{RT}) in Dogs: Additional Data

Report No.: 87474-1 MRID No.: 412343-04
Date: November 21, 1988

Other than stating that a cholinesterase NOEL was not determined and individual ophthalmological data were not submitted in the one-year dog feeding study with technical methamidophos (MRID No.: 00147938), Toxicology Branch/HED/EPA did not request additional data for this study which was evaluated in 1985. However, individual data on ophthalmological examinations were requested by Health Protection Branch/Health and Welfare Canada.

The registrants' response to Canada was also submitted to EPA (current submission), but "only as supplemental information".

Ophthalmological examination was performed on all dogs, before study initiation and at its termination. Nothing remarkable was observed at both time intervals. The dose levels of methamidophos used in this study were 0, 2, 8 and 32 ppm, expressed as an active ingredient.

The currently submitted data do not, therefore, affect the original evaluation of this study, namely:

> "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 2ppm (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)."

Raymond R. Loche 4/2/90

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5

Attachment II

Chronic Feeding/Oncogenicity Study of Technical Methamidophos (MONITOR^{RT}) To Rats: Additional Data

Report No.: 88687-1 MRID No.: 412343-03
Date: December 21, 1981

Other than stating that a cholinesterase NOEL was not determined in the rat chromic feeding/oncogenicity study with methamidophos (MRID No.: 00148452), Toxicology Branch/HED/EPA did not request additional data for this study which was evaluated in 1985. However, the following data were requested by Health Protection Branch/Health and Welfare Canada:

1. Gross and histopathology data for the 10 rats/sex/group sacrificed at 12 months.

2. Historical control data on organ weights of Fischer 344 rats submitted in such a format that:

   a. The time duration over which data were collected can be determined.

   b. The total collection time is broken down into increments so that any trends for changes in ranges with time can be assessed.

The registrants' response to Canada was also submitted to EPA (current submission), but "only as supplemental information".

Historical control data on organ weights were included in the original report on this study, although not in a format requested by Canada, and were considered by Toxicology Branch/HED during an evaluation of this study. Pathology of the satellite animals (10/sex/dose level sacrificed at 12 months) was not included in the original report because there was no evidence of induced toxicity or oncogenicity in the regular test groups (50 rats/sex/dose level).

The following dose-unrelated gross and histopathological findings predominated in the satellite animals:

Gross Pathology

- Eyes: Principally unilateral lesions were described as small, red or white, or deformed and were interpreted to result from the orbital bleeding technique used for blood sampling, in both sexes.

- Ovaries: Cysts.

- Uterus: Dilated or fluid-filled.

1

Histopathology (Non-neoplastic lesions)

- Eye: Cataracts, retinal degeneration, hemorrhages, keratitis, phthisis bulbi, synechia, degeneration of optic nerve and inflammation of harderian glands, in both sexes.
- Heart: Cardiomyopathy, mostly in males.
- Kidneys: Nephropathy, mostly in males.
- Prostate: Inflammation.
- Testes: Interstitial cell hyperplasia.
- Large Intestine: Mineralization of the mucosa, in both sexes.
- Liver: Hyperplasia of bile ducts and/or focal granulomatous inflammation, in both sexes.
- Lungs: Peribronchial inflammation, interstitial pneumonia and calcification, in both sexes.
- Pituitary Gland: Cysts and hyperplasia, in both sexes.
- Uterus: Endometrial stromal polyps.
- Ovaries: Cysts.

The above lesions were generally morphologically less extensive but similar to the type of lesions seen in the previously reported regular study.

The following neoplastic lesions were seen in rats sacrificed after one year on the study:

- Pituitary adenoma: In control males (1), 6 ppm females (1) and 54 ppm females (1).
- Preputial gland adenoma: In control males (1).
- Skull osteosarcoma: In 6 ppm males (1).
- Uterine endometrial stromal polyp: 1, 1, 1, 5 and 1 in the 0, 2, 6, 18 and 54 ppm groups, respectively.

The currently submitted gross and histopathology data (individual and summaries) for rats in the satellite group do not affect the original evaluation of this study, namely:

1. Technical methamidophos was not carcinogenic to Fischer 344 strain of rats at dose levels of 0, 2, 6, 18 or 54 ppm. 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.

Raystyna R. Loche 4/2/90

Roger Gardner 4-23-90

9

Attachment III

007891

Oncogenicity Study of Methamidophos Technical (MONITOR^{RT}) to Mice: Additional Data

Report No.: 87479-1 MRID No.: 412343-02
Date: December 21, 1988

Toxicology Branch/HED/EPA evaluated the above-cited mouse oncogenicity study in 1986 (Study No.: 80-332-01 and 87479; August 6, 1984; MRID No.: 00145579) and accepted it as Core-Guideline, requesting no additional data. However, pathology data on satellite animals were requested by Health Protection Branch/Health and Welfare Canada. Mice in the satellite groups (known also as replacement animals), 10/sex/dose level, were used for hematological determinations at 6 months and 1 year (week 53), when they were sacrificed. Since gross and histopathology data for the regular study CD-1 mice (50/sex/dose level) were included in the original report, Toxicology Branch/HED did not regard pathology data for the satellite mice as essential to the assessment of the oncogenic/carcinogenic potential of Methamidophos in this study.

The registrants' response to Canada was also submitted to EPA (current submission), but "only as supplemental information". The following dose-unrelated gross and histopathological findings predominated in the satellite mice:

Gross Pathology

- Eyes: Principally unilateral lesions (opaque or white eyes) were attributed to retrobulbar bleeding technique used in obtaining blood samples for hematological evaluations, in both sexes.
- Kidneys: Small renal cortical cysts, mostly in males.
- Ovaries and Uterus: Cysts.
- Stomach: Mucosal thickening or dilatation, in both sexes.

Histopathology (Non-neoplastic lesions)

- Eyes: Phthisis bulbi, retinal degeneration and harderian gland necrosis or inflammation, in both sexes.
- Kidneys: Lymphocytic inflammation and nephropathy, in both sexes.
- Liver: Lymphocytic/granulomatous inflammation, in both sexes.
- Lungs: Hemorrhage and lymphoid hyperplasia, in both sexes.

11

- Optic nerve: Degeneration, mostly in females.
- Ovaries: Cysts.
- Skull: Acute inflammation, in both sexes.
- Stomach: Epithelial hyperplasia and lymphocytic inflammation, in both sexes.
- Testes: Atrophy/degeneration and interstitial cell hyperplasia
- Uterus: Cystic endometrial hyperplasia.

The above lesions were generally morphologically less extensive but similar to the type of lesions seen in the previously reported regular study.

The following neoplastic lesions were seen in the satellite mice sacrificed at 12 months:

Organ*	Sex	Methamidophos (ppm) 0	1	5	25
Liver: Hepatocellular carcinoma	Males	2	1	0	1
Lungs: Bronchiolar adenoma	Males	1	0	1	0
	Females	0	1	0	2
Stomach: Carcinoma	Males	0	0	1	0
Thymus: Malignant lymphoma	Males	1	0	0	0
	Females	1	0	1	0

*In each instance, 10 tissues/sex/group were examined. The control male with malignant lymphoma in the thymus, which died after 9 months on the study, had also malignant lymphomas in every tissue examined.

The currently submitted gross and histopathology data (individual and summaries) for mice in the satellite group do not affect the original evaluation of this study, namely:

12

1. Technical methamidophos was not carcinogenic to CD-1 mice at dose levels of 0, 1, 5 or 25 ppm. Higher levels could not be used because the animals could not survive the toxic effects resulting from cholinesterase inhibition.

2. Based on decreased body weight gain and food consumption, a systemic NOEL was 5 ppm and LEL was 25 ppm.

Raymond R. Loche 4/2/90

R.Z. 4-23-90

13

Attachment IV

Reproductive Toxicity Study in Rats: Additional Data

Report No.: 88686-1 MRID No.: 412343-01
Date: December 9, 1988

The rat reproduction study (Accession No./MRID: 257632/00148455) was evaluated by Dynamac Corporation/Toxicology Branch (HED) in 1986 and classified as Supplementary because data, as reported, did not provide adequate information for assessing the reproductive toxicity of methamidophos and determining a reproductive NOEL. Of primary concern were reductions in reproductive performance at all dose levels, particularly in the F_0 females, when measured as a function of the number of sperm-positive females giving birth (see TABLE 6 from the original review; attached). In order to determine if these reductions were associated with events during fertilization, implantation or intrauterine death after implantation, individual animal reproduction data were requested. These data were to include the dates at the initiation of cohabitation, dates when plug or sperm in vaginal smears were found, and results of special examination procedures used to determine the pregnancy status of females whose uteri appeared to be nonpregnant.

The current submission includes most of the requested data, as well as the reproduction historical control data (see TABLE VII; attached) and a statement entitled "Rebuttal to the U.S. EPA's Technical Assessment of the Study" (attached).

According to the currently submitted mating data, most of the copulatory plugs and/or sperm-positive vaginal smears were observed within the first four days of cohabitation, in both generations (F_0 and F_1) and in all test groups. During the production of the F_1 and F_{2a} litters, the cohabitation was terminated after 11 days. During the production of the F_{2b} litters, the cohabitation was terminated after 10 days. Only one F_1 litter was produced in this study.

No special procedures/methods, such as immersion of uteri in a 10% solution of ammonium sulfide, were used to visualize possible implantation sites. Inseminated females that did not produce litters were considered as nonpregnant by the testing laboratory. Without these data, it is impossible to determine if the nonpregnancy of inseminated females was associated with fertilization, implantation or death after implantation.

Mobay Corporation (the registrant) disagrees with the reviewers (Dynamac Corporation/EPA) that a NOEL for reproductive toxicity was not established in this study. The major reasons for the disagreement were as follows:

1. The statistically significant ($p \leq 0.05$; Fisher's exact test) reduced fertility of the low-dose and mid-dose F_0 females was due to the unusually high fertility index of the F_0 control group (i.e., 100%).

15

2. The 100% fertility index was the highest ever obtained by Mobay for the F_0 control group in reproduction studies with the same strain of rats (CrL:CD), conducted in their laboratory.

3. According to the historical control data, the fertility indices were highly variable for the F_0 females and ranged from 41.9% to 96.2% in the six submitted "contemporary" studies, conducted in Mobay laboratory. Similar variability was observed in the F_1 generation during the production of the F_{1b} pups, but there was less variability during the production of the F_{2a} pups.

4. The fertility indices for all F_0 methamidophos-treated females fell within the range of the historical control values.

5. The decreases in fertility of the F_0 methamidophos-treated females were dose-unrelated and could not, therefore, be attributed to treatment. Fertility indices of 65.2, 76.0 and 62.5% were observed for F_0 females treated at dietary levels of 3, 10 and 33 ppm, respectively, of methamidophos.

6. If the reduction in fertility indices, observed in the F_0 females, were a treatment-related effect, one would have expected it to have occurred also in the next generation. However, there was no statistically significant reduction in the percentage of sperm-positive, low- and mid-dose, F_1 females delivering pups.

Considering the currently submitted additional data, especially the reproductive historical control data, this study is now being upgraded as follows:

Classification: Core-Minimum

Reprod. NOEL = 10 ppm
LEL = 33 ppm (decrease in the number of sperm-positive females giving birth)

Develop. NOEL = 10 ppm
LEL = 33 ppm (decreases in pup viability and body weight during lactation)

Syst. NOEL = 10 ppm
LEL = 33 ppm (decreased body weights of males and females during premating and of females during lactation)

Raymond R. Locke 4/2/90

Rose Lemen 4-23-90

14

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.

17

TABLE VII

Mobay Corporate Toxicology Laboratories
Reproduction Historical Control Data
Crl:CD (Sprague-Dawley) Rats

	Study Number							
	82-671-01 (Methamidophos)	81-671-01	83-671-01	85-671-01	85-671-02	87-671-01 & 02		Range
							MIBK[c]	
Mating to Produce F1a Pups								
Number of Dams	26	26	31	35	35	35	35	
Number Inseminated	25	26	29	31	34	35	35	
Number of Litters	25	25	26	13	29	31	30	
Fertility Index (%)[a]	100	96.2	89.7	41.9	85.3	88.6	85.7	41.9-100
Mating to Produce F1b Pups								
Number of Dams	--[b]	--[b]	29	30	31	33	33	
Number Inseminated	--	--	25	25	25	33	31	
Number of Litters	--	--	11	13	8	27	26	
Fertility Index (%)	--	--	44.0	52.0	32.0	81.8	83.9	32.0-90.3
Mating to Produce F2a Pups								
Number of Dams	26	26	30	25	29	35	35	
Number Inseminated	22	24	26	24	28	33	34	
Number of Litters	16	24	22	18	25	26	31	
Fertility Index (%)	72.7	100	84.6	75.0	89.3	78.8	91.2	72.7-100
Mating to Produce F2b Pups								
Number of Dams	26	26	29	24	25	35	35	
Number Inseminated	20	25	20	23	24	30	27	
Number of Litters	16	24	13	12	20	23	20	
Fertility Index (%)	80.0	96.0	65.0	52.2	83.3	76.7	74.1	52.2-96.0

a. Fertility Index = $\frac{\text{number of inseminated females producing litters}}{\text{number of inseminated females}} \times 100$

b. Only 1 litter produced by F_0 animals per study protocol.

c. This group served as a vehicle control group and received a diet containing 50 ppm of methyl isobutyl ketone (MIBK).

Rebuttal to the U.S. EPA's Technical Assessment of the Study

We disagree with the reviewer's (Dr. G. Millicovsky, Dynamac Corporation) opinion that a NOEL for reproductive toxicity was not established in this study. The reviewer's opinion was based on the fact that at all treatment levels there was a statistically significant reduction ($p \leq 0.05$; Fisher's exact test) in the percentage of sperm-positive F_0 females delivering F_1 pups (i.e. fertility index). We concur with the reviewer's statistical analyses of the data, however, we do not agree with his interpretation of the data. There is good evidence to demonstrate that the statistically significant effects seen at the low and mid dose levels are not biologically relevant.

The statistically significant reduced fertility indices for F_0 females of the low and mid dose is due to the unusually high fertility index of F_0 control females (i.e. 100%). Out of six reproduction studies conducted at Mobay, this is the highest fertility index ever obtained for F_0 control animals. Table VII demonstrates that for untreated animals this parameter is highly variable ranging from 41.97% to 100%. The following examples are discussed merely to illustrate that the statistical significance is an artifact produced by the random occurrence of an unusually high fertility index in control animals. The Fisher's exact test would not have been statistically significant for low and mid dose animals if a fertility index of ≤88% would have been obtained. Eighty-eight percent is 4% greater than the historical control mean fertility index for F_0 animals in our laboratory. Furthermore, if one uses the fertility indices obtained for the first and second mating of F_1 control animals in this study (i.e. 72.7% and 80.0%, respectively), Fisher's exact test for the fertility indices of low and mid dose F_0 animals are not statistically significant.

The fertility indices for all F_0 treated animals fell within the range of contemporary historical control values for our laboratory. Fertility indices of 65.2, 76.0 and 62.5% were observed for F_0 females treated at dietary levels of 3, 10, and 33 ppm, respectively. These values all fall within the historical control range which, as shown in Table VII, ranges from 41.9 to 100% for F_0 females. This, coupled with the fact that the decreases in fertility indices for treated groups were not dose-related, provides good evidence that the observed effects were not related to treatment.

More importantly, if this was a treatment-related effect, one would have expected it to have occurred in the next generation as well. However, there was no statistically significant reduction in the percentage of sperm-positive, low and mid-dose, F_1 females delivering pups. This is true for both the first and second mating of the F_1 animals. These data, taken collectively, demonstrate that the statistically significant effects at the low and mid-dose were not biologically relevant.

19

Attachment V

007891

Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 3/19/90
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Roger Gardner, Acting Section Head
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Irving Mauer, Geneticist 3/21/90
Tox. Branch I/IRS (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Chromosome Aberrations In Vivo (mouse bone marrow cells)

Tox. Chem. No.: 378A

MRID No.: 412343-06

TEST MATERIAL: Technical methamidophos; purity (methamidophos or active ingredient, a.i., content): 74.4%; off-white powder readily soluble in water or ethanol; received by the testing laboratory as clear liquid.

SYNONYMS: Compound SX-1244 (used only in this study; not a universally accepted synonym/common name.)

STUDY NUMBER(S): MRI-176-CCC-82-56

SPONSOR: Valent U.S.A. Corporation/Chevron Chemical Company

TESTING FACILITY: EG & G Mason Research Institute, Worcester, MA

TITLE OF REPORT: In Vivo Cytogenetics Study in Mice. Methamidophos Technical (SX-1244)

AUTHOR(S): H. J. Esber

REPORT ISSUED: November 18, 1983

CONCLUSIONS:

Classification: Acceptable

Technical methamidophos was not clastogenic to bone marrow cells of CD_1 male and female mice. In the major cytogenetic study, mice were treated orally (gavage) with single doses of technical methamidophos ranging from 0.8 to 8.1 mg/kg (0.6 to 6.0 mg/kg a.i.) and were sacrificed at 6, 24 and 48 hours after treatment. In the supplemental cytogenetic study, mice were treated with single oral doses of technical methamidophos ranging from 8.1 to 16.1 mg/kg (6-12 mg/kg a.i.) and were sacrificed at 24 hours after treatment. The incidence of total chromosomal aberrations was similar in the methamidophos-treated and distilled water-treated (negative control) groups. Positive findings were obtained with cyclophosphamide, a known clastogen.

21

The doses used in the cytogenetic studies were based on an acute oral LD_{50} study with the same strain of mice and the same batch of technical methamidophos. The LD_{50} and maximum tolerated dose (MTD) in that study were 15.2 mg/kg (a.i.) and 6.6 mg/kg (a.i.), respectively, for the female mice. The LD_{50} and MTD for the male mice were not calculated because of insufficie t data.

Toxic signs observed in the acute oral LD_{50} and cytogenetic studies were those typical of cholinesterase inhibition.

EXPERIMENTAL PROCEDURES

This study includes actually two cytogenetic studies, one in which technical methamidophos was tested at doses ranging from 0.8 to 8.1 mg/kg and another in which doses ranging from 8.1 to 16.1 mg/kg were used. The testing laboratory referred to the first study as "original" and to the second study as "supplemental". An acute oral toxicity study (to determine doses for the original cytogenetic study) and a range-finding study (to determine doses for the acute oral study) were also conducted.

Range-Finding Study

Male and female mice, 2/sex/dose, received single oral doses of technical methamidophos a: i then were observed daily for 14 days. The doses of technica methamidophos used were 0, 4.0, 12.1, 36.3 and 108.9 mg/kg. Since technical methamidophos (Compound SX-1244) contained 74.4% on a weight basis of methamidophos (active ingredient or a.i.), the above doses correspond to 0, 3, 9, 27 and 81 mg/kg, respectively, of a.i.

Acute Oral LD_{50} Study

Male and female mice, 5/sex/dose, received single oral doses of methamidophos and then were observed daily for 14 days. The doses of technical methamidophos used were 0, 12.1, 18.1, 27.2 and 40.8 mg/kg and were based on the range-finding study. These values correspond to 0, 9.0, 13.5, 20.2 and 30.4 mg/kg, respectively, of a.i. (methamidophos alone). The mice were weighed just before treatment, after one week of observation and at the termination of the study. All mice were necropsied.

In Vivo Cytogenetic Study (Original)

Male and female mice, 12/sex/dose, were given single oral doses of technical methamidophos and were sacrificed, 4/sex/dose/time interval, at 6, 24 and 48 hours following treatment. The doses of technical methamidophos used were 0.8, 2.7 and 8.1 mg/kg, corresponding to 0.6, 2.0 and 6.0 mg/kg, respectively, of methamidophos (a.i.). These doses were based on the results of the acute oral study in which the maximum tolerated dose (MTD) for females was 6.6 mg/kg and for males

< 6.6 mg/kg (not established definitively). The negative control group, 12/sex/dose, received distilled water, a vehicle for both technical methamidophos and cyclophosphamide (positive control). Mice in the positive control group, 4 males and 4 females, were sacrificed at 24 hours after treatment. Cyclophosphamide, 25 mg/kg, was administered intraperitoneally.

Approximately 2 hours before sacrifice, an intraperitoneal injection of colchicine in Hank's balanced salt solution (1.2 mg/kg) was given each mouse to inhibit mitosis. The animals were sacrificed by cervical dislocation following carbon dioxide anesthesia.

Bone marrow of both femurs was aspired into a prewarmed (37° C) Hank's balanced salt solution. After centrifugation, cells were suspended in 0.075 M potassium chloride, treated with Carnoy's fixative, placed on microscopic slides, stained in Giemsa Stain, rinsed with water, acetone, acetone:xylene and xylene, and dried at 35-40° C for a minimum of 2 hours before storing.

Fifty metaphase spreads per animal were analyzed, when possible. Cytogenetic aberrations (chromatid and chromosome breaks and other markers such as rings, deletions, fragments, and exchanges) were scored. Gaps were also scored and reported but were not considered as chromosomal damage. The mitotic index on each animal was determined.

The animals were observed daily for toxic signs and were weighed upon receipt, prior to dosing and before sacrifice.

In Vivo Cytogenetic Study (Supplemental)

This study was conducted like the original study, except that the doses of technical methamidophos used were 0, 8.1, 12.1 and 16.1 mg/kg. These values correspond to 0, 6, 9 and 12 mg/kg of methamidophos (a.i.). There were 4 males and 4 females per dose and all were sacrificed at 24 hours after treatment. This study was conducted shortly after the original study and a positive control was not used.

Sections of Experimental Protocol Applicable to All of the Above Studies

The CD_1 strain of mice was used. The animals were obtained from Charles River Laboratories, Inc., Wilmington, Massachusetts, or Portage, Michigan, and weighed 25-35 g (males) and 20-30 g (females) at the initiation of treatment. The animals were:

1. Acclimated for 7 days, at the end of which 2 mice of each sex, randomly selected, were sacrificed and examined by a veterinary pathologist for intercurrent disease and nematode infestation.

23

2. Housed, by sex, 5/cage in the acute oral LD_{50} study and 4/cage in the cytogenetic studies, at temperature of 22.2-25° C, relative humidity of 44-64% and 12-hour light/dark cycle.

3. Identified by ear punch.

4. Assigned to cages on a weight basis.

5. Fed unrestricted amounts of food (NIH 07 open diet mash; Zeigler Bros., Inc., Gardners, PA) and tap water.

6. Fasted for 4 hours before treatment.

7. Dosed by gavage, using 5 ml of a methamidophos solution/kg of body weight. Other dosing solutions (distilled water and cyclophosphamide) were also administered in the same volume. Dosing and volume were based on the individual body weights determined on the day of treatment. The same batch of methamidophos was used in all four studies.

Statistical Analyses

Terminal body weights and the mean number of bone marrow cells with aberrations were analyzed by comparing the methamidophos-treated and positive control groups with the negative control group. The following formulae were used for calculating the standard deviation, standard error and t-test:

1. $$S.D. = \sqrt{\frac{(x-\bar{x})^2}{n-1}}$$

2. $$S.E. = \frac{S.D.}{\sqrt{n}}$$

3. $$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad ; d.f. = n_1 + n_2 - 2$$

RESULTS

Stability of the Test Material

Technical methamidophos (a clear liquid) was stored upon receipt in the dark at room temperature because it crystallized out of solution when stored frozen and then thawed. Protocol provided by the sponsor indicated that Compound SX-1244 (technical methamidophos) was 74.4% methamidophos (a.i.) and "relatively stable at ambient temperatures", but the actual stability data were not submitted. Dosing solutions were prepared just before use.

In Vivo Cytogenetic Studies

Toxic signs observed in male and female mice receiving 8.1 mg/kg of technical methamidophos (6 mg/kg a.i.) included inactivity, clear exudate from both eyes and ataxia, for a few hours after administration. Mice treated with 12.1 mg/kg of technical methamidophos (9 mg/kg a.i.) were ataxic, haunchy and shaking and those treated with 16.1 mg/kg of technical methamidophos (12 mg/kg a.i.) were also convulsive. No signs of toxicity were observed in the low-dose (0.6 mg/kg a.i.) and mid-dose (2 mg/kg a.i.) methamidophos-treated groups and in the positive and negative control groups. There were no deaths in any group.

Technical methamidophos had no clastogenic potential in this study, even at the dose of 12 mg (a.i.)/ kg or about twice the MTD. The incidence of total chromosomal aberrations observed, aberrations including gaps or aberrations excluding gaps, was similar in the methamidophos-treated and negative control groups. However, compared with the negative control group, statistically significant increases in chromatid breaks were noted in the cyclophosphamide-treated males ($p < 0.007$) and females ($p < 0.07$). Mitotic indices* in the treated and negative control groups were similar. (See Attachment I for details).

Acute Oral LD_{50} Study

Toxic signs were observed in all of the methamidophos-treated groups, but not in the vehicle control group. Signs observed in the treated groups included clear or red exudate from both eyes, excess salivation, lethargy, tremors, dyspnea, convulsions and moribundity.

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*The mitotic index is defined as the percentage of cells undergoing mitosis and calculated as follows:

Percent Mitotic Index = (Number of mitotic figures/500 cells analyzed) x 100

Gross necropsy revealed no abnormalities in the vehicle controls and low-dose (9 mg/kg a.i.) groups, and clear exudate from eyes and mouth and irregular liver patches in some mice from the remaining groups.

There were no deaths in the vehicle control and low-dose groups, and no survivors in the 20.2 mg/kg (a.i.) male and 30.4 mg/kg (a.i.; HDT) male and female groups.

The LD_{50} and 95% confidence limits, and MTD for the female mice were as follows:

LD_{50} = 15.2 (11.8-19.7) mg/kg, expressed as a.i., and determined using the Cornfield-Mantel Method**

MTD = 6.6 mg/kg (a.i.)

The LD_{50} and MTD for the male mice were not calculated because of insufficient data. Of the 4 data points obtained for the male mice, 3 were either 0% or 100% mortality.

Range-Finding (For the Acute Oral LD_{50}) Study

Toxic signs observed in the range-finding study at doses of 3 and 9 mg/kg (a.i.) were similar to those observed in the LD_{50} study and there was no mortality. At higher doses (27 and 81 mg/kg; a.i.), paralysis was also observed and all animals died.

COMMENTS

In general, this study is well planned and reported. However, the following ambiguities/errors should be noted:

1. The purity of technical methamidophos (that is, its a.i. or methamidophos content) was reported as 73.5% on page i and 74.4 % on page 31. The value of 74.4% "fits" well when doses are converted from technical methamidophos to a.i., whereas the value of 73.5% "fits" less well.

2. There is an error in TABLE 16, page 27 of the submission. According to that table, male mice in the supplemental cytogenetic study, treated with 6, 9 and 12 mg/kg of methamidophos (a.i.), were sacrificed at 6, 24 and 48 hours following treatment, respectively. Actually, all mice in the supplemental study were sacrificed at 24 hours after exposure.

**Cornfield and Mantel, 1959. Cornfield-Mantel Modification of Karber's Method. JASA 45: 193-210.

Quality Assurance Statement was submitted indicating that the studies were inspected as follows: acute oral LD_{50} , on 9/15/82; the original cytogenetic study, 4 times during 10/11-12/6/82; and the supplemental cytogenetic study, on 8/29 and 11/18/83.

27

Attachment I

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 29 through 42 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

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 3/26/90
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Roger Gardner, Acting Section Head Roger Gardner 4-23-90
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Irving Mauer, Geneticist 5/30/90
Tox. Branch I/IRS (H7509C)

007891

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - DNA Damage/Repair in Vitro (UDS/rat hepatocytes)

Tox. Chem. No.: 378A
MRID No.: 412343-05

TEST MATERIAL: Monitor Technical (Methamidophos); purity: 71.2%; reference number: 77-297-149; clear colorless liquid, received on 12/8/87, stored at room temperature and diluted with dimethylsulfoxide (DMSO) for testing.

STUDY NUMBER(S): MBA: T5844.380
Chevron: 99172

SPONSOR: Mobay Corporation, Stilwell, KS

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes

AUTHOR(S): Rodger D. Curren

REPORT ISSUED: October 24, 1988

CONCLUSIONS:

Classification: Acceptable

Monitor Technical, at concentrations ranging from 0.001 µl (0.7µg) to 1.0 µl (712 µg)/ml of assay medium, did not induce unscheduled DNA synthesis (UDS) in rat primary hepatocytes. Concentrations of Monitor greater than 1 µl/ml were too cytotoxic to be evaluated for UDS. 7,12-Dimethylbenz(a)anthracene (DMBA; 3 and 5 µg/ml) served as the positive control.

EXPERIMENTAL PROCEDURES

The laboratory phase of this study was conducted from December 20, 1987, to March 9, 1988, and included a preliminary cytotoxicity test. Hepatocytes were isolated by the procedure of Williams et al. (1); cytotoxicity and unscheduled DNA synthesis

1. Williams, G.M. et al. In Vitro 13:809-817, 1977.

43

(UDS) assays were performed by the procedure of Williams (2); and the obtained data were evaluated also by the procedures of Williams (3,4).

Hepatocytes from an untreated rat, 5 x 10^5 viable cells per culture plate, were allowed to attach to coverslips during an incubation in Williams Medium E (WME), supplemented with fetal bovine serum, L-glutamine and gentamicin, at 37 ± 1° C for 90-150 minutes. The cultures, washed and resuspended in serum-free WME, were then exposed for 18-20 hours to ^{3}H-thymidine (10 µCi/ml) together with 9 concentrations of Monitor, ranging from 0.001 to 10 µl (0.7-7120 µg*)/ml of assay medium. The coverslips were then washed in serum-free WME and prepared for scoring silver grains (a measure of UDS) according to the referenced (above) procedures. Nuclear grains were counted in 50 cells, where possible, in random areas on each of the three coverslips used per concentration of Monitor. Dimethylsulfoxide (DMSO) was a solvent/diluent for both Monitor and 7,12-dimethylbenz(a)anthracene (DMBA; 3 and 5 µg/ml), a positive control.

The UDS assay included a simultaneous cytotoxicity test. In parallel with the UDS test plates, 3 cultures per concentration were tested with Monitor and control compounds, but the coverslips and ^{3}H-thymidine were omitted. Following the 18-20-hour incubation, an aliquot of each culture medium was centrifuged and the level of lactic dehydrogenase (LDH) in the supernatant determined. Cytotoxicity was determined from the amount of LDH which was released by the hepatocytes into the culture medium. The relative toxicities** were obtained by comparing the LDH levels and survival of hepatocytes in the treated cultures with those in the negative control (solvent) cultures. LDH is a membrane-bound enzyme and its release from membrane increases with loss of the cell membrane integrity. Microscopic examination of the hepatocyte cultures was, therefore, also used in the assessment for cytotoxicity.

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2. Williams, G.M. Cancer Research 37:1845-1851, 1977.
3. Williams, G.M., The detection of chemical mutagens/carcinogens by DNA repair and mutagenesis in liver culture. In: Chemical Mutagens, Vol. VI, F.J. DeSerres and A. Hollaender, eds. Plenum Press, New York, pp. 71-79, 1979.
4. Williams, G.M., Carcinogen-induced DNA repair in primary rat liver cell cultures, a possible screen for chemical-carcinogens. Canc. Lett., 1:231-237, 1977.

*In the submitted report, concentrations of Monitor Technical were expressed always as µl/ml of assay medium. Concentrations of Monitor in µg/ml were calculated by KKL, the toxicologist who evaluated this study, using purity of Monitor = 71.2%.

**Relative toxicity = Corrected LDH/100% LDH Control
Corrected LDH = Average LDH - Solvent Control LDH
100% LDH Control = Amount of corrected LDH activity released by exposure to 1% Triton

44

Concentrations of Monitor tested in the UDS assay were based on the results of a preliminary cytotoxicity study in which 10 concentrations of Monitor, ranging from 0.0003 to 10 µl (0.2-7120 µg)/ml of assay medium and two culture plates per concentration were used. Concentrations of Monitor causing 50-90% and 0-20% relative cytotoxicity were regarded as maximum and minimum, respectively, to be used in the UDS assay.

Adult male Sprague-Dawley rats were used in all of the above assays. The animals were obtained from Charles River Laboratories, Inc. (location not specified), acclimated for at least one week, given unrestricted amounts of food (Purina Certified Rodent Chow) and tap water, and sacrificed by inhalation of methoxyflurane (metofane). The livers were perfused first with 0.5 mM EGTA solution and then with collagenase solution before they were removed for the isolation of hepatocytes.

Criteria Used for Evaluation of Test (UDS) Results

Means, standard deviations and percent survival/cytotoxicity were calculated using the LOTUS 1-2-3 program on an IBM PC or compatible computer. If the mean net nuclear count was increased by at least five counts over the solvent control, this increase for a particular concentration tested was regarded as significant. A test compound was considered positive if it induced a concentration-related response and at least one concentration produced a significant increase in the mean net nuclear grains when compared to that of the solvent control. In the absence of the concentration response, a test compound which showed a significant increase in the mean net nuclear grain count in at least two successive concentrations was considered positive. If a test compound showed a significant increase in the net nuclear grain count at one concentration without any concentration-dependent response, the test compound was considered to have a marginal positive activity. The test compound was considered negative if no significant increase in the net nuclear grain counts at any dose level was observed.

RESULTS

Analysis of Monitor Solutions Used in the UDS Assay

Test solutions were prepared just before use. Two samples of the test solution containing the highest nominal concentration of Monitor (100 µl/ml) and the solvent control were saved by the testing laboratory for concentration analysis by the sponsor. However, the original solutions were misplaced and replacement solutions were prepared using procedures identical to those used to prepare the original test solutions. According to the sponsor, the average concentration of Monitor in the two samples

45

analyzed was 132% of the nominal concentration. The limit of reliable measurements by the gas chromatographic procedure used for analysis was 1.0 µg/ml. No data were available on the analytical concentrations of Monitor in other test solutions and on the stability of Monitor under the assay conditions.

Cytotoxicity

In the preliminary cytotoxicity test, measurements of LDH, released by hepatocytes, indicated relative toxicities of 49% and 37% at Monitor concentrations of 10 µl (7120 µg)/ml and 3 µl (2136 µg)/ml, respectively. The remaining eight concentrations of Monitor tested, 0.003 µl (0.2 µg)/ml to 1.0 µl (712 µg)/ml, caused relative toxicities ranging from 0 to 15%, in a concentration-unrelated manner. Based on these findings, the highest concentration chosen for the UDS assay was 10 µl/ml. (For details, see ATTACHMENTS, TABLE 1.)

Measurements of LDH levels in the repeated cytotoxicity test, conducted in parallel with the UDS assay, indicated relative toxicities of 50% and 73% at Monitor concentrations of 10 µl (7120 µg)/ml and 3 µl (2136 µg)/ml, respectively. The remaining seven concentrations of Monitor tested, 0.001 µl (0.7 µg)/ml to 1.0 µl (712 µg)/ml, were essentially nontoxic (LDH levels were in all instances lower than those in the solvent controls). Microscopic examination of the hepatocyte cultures indicated toxicity at 10, 3 and 1 µl/ml, with normal morphology at lower concentrations of Monitor.

Based of the measurements of LDH levels, the relative toxicities for DMBA (positive control) were 6 and 20% at concentrations of 3 and 5 µg/ml, respectively. (For details regarding parallel cytotoxicity test, see ATTACHMENTS, TABLE 2.)

Unscheduled DNA Synthesis

Monitor Technical was evaluated at concentrations of 0.001, 0.003, 0.01, 0.03, 0.3 and 1.0 µl.ml of assay medium, corresponding to 0.7, 2.1, 7.1, 21.4, 213.6 and 712.0 µg/ml, respectively. Monitor concentrations of 3 µl (2136 µg)/ml and greater were too cytotoxic to be evaluated for UDS. Hepatocytes exposed to 0.1 µl of Monitor/ml could not be evaluated due to poor slide preparation.

Monitor did not cause a significant increase in the mean number of net grain counts (i.e., an increase of at least 5 counts over the control) at any concentration that could be evaluated for UDS. There were no hepatocytes with 5 or more net nuclear grains in the solvent control assays, whereas the percentage of these hepatocytes in the Monitor-containing assays ranged from 0 to 2, in a concentration-unrelated manner. However, cultures treated with 3 and 5 µg/ml of DMBA (positive

46

control), contained, respectively, 66 and 70% of hepatocytes with 5 or more nuclear grains. (For details regarding UDS, see ATTACHMENTS, TABLE 3.)

The author concluded that Monitor Technical was not genotoxic when tested for induction of UDS in rat primary hepatocyte cultures. In contrast, DMBA induced the expected positive response. TB/HED agrees with these conclusions.

COMMENTS

In general, this study is well planned and reported. However, the following ambiguities/deficiencies should be noted:

1. According to page 6 of the submission, Monitor Technical (Reference No.: 77-297-149), used in the UDS assay, had a purity of 71.2%. According to page 31 of the same submission, Methamidophos (Monitor; Reference No.: 77-297-149), used as a standard in the gas chromatographic analysis of test solutions, had a purity of 72.9%. Since the reference numbers are the same, are these two compounds actually the same? If so, why are the purities different? A comment from the registrant/testing laboratory is required.

2. Concentrations of Monitor Technical in testing ("dosing") solutions were reported as µl/ml of assay medium, which is useless for comparison (with other studies) purposes. Concentration reported in terms of molarities or as µg/ml would have been more meaningful.

3. Monitor Technical was not tested for stability under assay conditions.

4. Test solutions were not analyzed for the concentrations of Monitor. Although a solution containing the highest nominal concentration of Monitor (100 µl/ml) was analyzed, it was not the solution which was used in the assays, but one "identically prepared." Also, since the sensitivity of the analytical (gas chromatographic) method used was 1.0 µg/ml, other test solutions could have been analyzed.

In summary, although this UDS study contains a few ambiguities and deficiencies, they are not serious enough to reject the study or even to classify it as supplementary. This study is, therefore, acceptable.

Quality Assurance Statement was submitted indicating that this study was inspected 5 times during 12/18/87 - 10/28/88.

47

ATTACHMENTS

4A

Methamidophos toxicology review

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___ Identity of product inert ingredients

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