6-20-89

CASWELL FILE

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

JUN 20 1989

007262

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 239-2471 - Methylthioacetate (Contaminant of Acephate): Review of Acute Oral and Dermal Toxicity Studies in Rats and Rabbits, Acute Inhalation Toxicity in Rats, Subchronic Dermal Toxicity Studies in Rats (21 days) and Rabbits (21- and 90-day studies); Studies to Assess for Possible Effects of Methylthioacetate on the Optic Nerve and/or Optic Chiasma and Ocular Function; Review of Mutagenicity/Genetic Toxicity Studies

TOX Chem Nos.: 2A (Acephate)
584D (Methylthioacetate)
TOX Project No.: 8-0556
Record No.: 214636
MRID Nos.: 405048-33 thru -43

FROM: John Doherty 6/2/89
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: William H. Miller, PM 16
Insecticide Rodenticide Branch
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THROUGH: Robert Zendzian 6/2/89 6/9/89
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Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Background

The Chevron Chemical Co. has submitted a series of acute and subchronic toxicity studies with rats and rabbits and mutagenicity/genetic toxicity studies with the chemical methylthioacetate (MTA), a contaminant of the insecticide acephate, in response to an earlier request from Toxicology Branch (TB, refer to J. Whalan review dated September 5, 1985 for EPA File No. 239-2471).

The purpose of the acute and subchronic studies with rats and rabbits was to further assess the potential of MTA to affect the eye (functional impairment), optic nerve and optic chiasma (histological changes). In the first of two studies previously reviewed by TB (refer to J. Whalan review above), which tested dose levels of about 2 mg/kg of MTA (applied dermally in gauze patches), "nonreversible diminution or absence of pupillary light reflex and blindness caused by gliosis, macrophage accumulation, malacia, and papilledema of the optic nerve, optic tract, and brain between the optic nerve and pituitary" resulted.

In the second study it was demonstrated that MTA affected the optic nerve of rabbits following a single dermal application of 250 mg/kg. In particular, according to J. Whalan's review "Histopathological lesions included gliosis, malacia, and macrophage accumulation in the optic nerve. Curiously, ophthalmic examination failed to find anomalies."

In order to further assess for the potential of MTA to affect the optic system of test animals acute oral and dermal toxicity studies with rats and rabbits, an acute inhalation study with rats, and subchronic (21-day) dermal toxicity studies with rats and rabbits and a subchronic (90-day) dermal toxicity study with rabbits were submitted.

In addition to the problem of potential ocular system effects of MTA, this chemical was also found to be mutagenic in a mouse lymphoma assay. The bacterial mutagenicity study (Ames test), which was submitted earlier, was found to be UNACCEPTABLE. Additional mutagenicity studies were requested (refer to Whalan review above). In response to this request the registrant has submitted three mutagenicity or genetic toxicity studies.

The studies submitted were reviewed and the following comments apply.

TB Comments

Part I: Regulatory Considerations

1. Overall Conclusion Regarding MTA and the Optic System

   TB has determined based on the information provided thus far that MTA treatment both orally and dermally results in optic tract (optic nerve and chiasma) lesions and functional effects in the eye of rabbits.

A NOEL for these effects is set at 50 mg/kg/day based on the 21-day rabbit dermal toxicity study. [Note: In this study two of the rabbits dosed with 50 mg/kg/day displayed an apparent delayed pupillary response on 1 or 2 days but this was not considered by TB to be significant enough to assign 50 mg/kg/day as a definite effect level.]

With regard to assessing for potential human health effects resulting from MTA contamination of acephate (or Orthene) the following table showing several margins of safety (MOSs) resulting from registered uses of acephate has been prepared.

Use	Daily Exposure to Acephate (mg/kg/day)[1]	Daily Exposure to MTA (mg/kg/day)[2]	MOS
Homeowner	0.17	0.00017	294118
Greenhouse (Ornamental)	0.34	0.00034	147059
Residential PCO	0.41	0.00041	121951
Commercial PCO	0.13	0.00013	384616
Tobacco-Foliar	0.023	0.000023	2173913
Tobacco-Transplant	0.047	0.000047	1063830
Turf Grass	0.83	0.00083	60241

[1]The daily exposure data related to acephate was obtained from the Exposure Assessment Branch memorandum dated June 23, 1988 prepared by Curt Lunchick.

[2]The daily exposure of MTA to humans was estimated by multiplying the daily exposure to acephate by 0.001 or 0.1 percent. [redacted]

It is assumed by TB that the ratio of MTA to acephate remains constant during usage. TB considers it unlikely that humans will be exposed to a significantly higher proportion of MTA.

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

The MOSs determined above for MTA are considered by TB to be such that users of acephate are not at significant risk for any potential optic system effects of MTA.

No additional studies are required at this time to further assess the potential of MTA to affect the optic system of experimental animals.

2. Mutagenicity and Genetic Toxicity Testing

Two of the three mutagenicity/genetic toxicity studies submitted were reviewed and determined to be ACCEPTABLE (the Salmonella assay and the mouse micronucleus assay). The third study, the chromosome aberration study in mouse bone marrow was determined to be UNACCEPTABLE. When these studies are taken together with studies previously submitted (a Salmonella assay and a mouse lymphoma assay which was considered positive), the registrant has satisfied the data requirements for mutagenicity Categories 1: gene mutation, and 2: structural chromosome aberration. Note: Although the chromosome aberration study with rat bone marrow was considered UNACCEPTABLE, an ACCEPTABLE mouse micronucleus study is available to satisfy the requirement for a structural chromosome aberration study.

In order to complete the requirements for mutagenicity/genetic toxicity testing, the registrant must provide an ACCEPTABLE study from Category 3 (other genotoxic effects).

When this study is completed, TB will review all data (including the mouse lymphoma study which was determined to be positive) and then determine if further testing will be required to pursue a mutagenicity risk assessment, if considered appropriate.

Part II: Discussion of the Toxicological Problem of MTA Induction of Functional and Histological Effects in Test Animals.

1. Toxicity Testing in Rabbits

a. Acute Toxicity Testing (Oral and Dermal) in Rabbits

The observation that MTA induces lesions in the optic system as alleged in earlier studies (refer to J. Whalan review above) was again apparent in both the rabbit acute oral and dermal toxicity studies.

None of the rabbits in the control groups in the acute oral or acute dermal toxicity studies were reported as developing either functional effects in the eye or pathological conditions in the optic nerve or optic chiasma.

In the acute oral toxicity study, 8 of the 27 rabbits examined in the groups dosed with either 150 or 250 mg/kg of MTA developed lesions in the optic nerve and/or optic chiasma which were described as chronic granulomatous inflammation, vacuolar change, gliosis and/or necrosis. The functional changes noted were mostly in the rabbits dosed with 250 mg/kg and above and included mydriasis, nystagmus and absent or delayed pupil response.

In the acute dermal toxicity study, 16 of the 32 rabbits examined in the groups dosed with either 175 or 250 mg/kg of MTA developed lesions in the optic nerve and/or chiasma which were described as chronic granulomatous inflammation, necrotic inflammation, necrosis or vacuolar change. The functional effects in the eye were described as mydriasis and delayed or absent pupil response which persisted to day 14 and delayed palpebral response which was reported to persist for 1 day only.

As an acute dermal toxicity study, the rabbit study was determined to be SUPPLEMENTARY. No acute dermal LD_{50} was established and since rabbits that were dosed at 175 and 250 mg/kg died, it is possible that the LD_{50} is less than 200 mg/kg and that MTA requires classification as Toxicity Category I. A third acute dermal toxicity study is not required since MTA is not a pesticide requiring label statements.

b. Subchronic (21- and 90-Day) Testing in Rabbits

The 21-day dermal toxicity study with rabbits was determined to be CORE GUIDELINE. This study was assigned a NOEL of 50 mg/kg/day for effects on the

optic system. The LEL for effects on the optic system was determined to be 100 mg/kg/day based on evidence of delayed pupillary responses and lesions (chronic granulomatous inflammation, neuritis, and/or necrosis) in the optic nerve and/or optic chiasma were present in some of the treated rabbits.

The 90-day dermal toxicity study with rabbits was determined to be INVALID because many of the rabbits died apparently as the result of infectious "mucoid enteritis." The rabbits in all groups dosed with MTA in this study had higher death rates than the untreated controls although the control rabbits demonstrated clear signs of having had this infection. None of the surviving rabbits treated with MTA developed optic tract lesions or functional impairment of the ocular system. The apparent functional effects (delayed pupillary response) of MTA noted at 50 mg/kg/day in the 21-day dermal toxicity study were not noted at 60 mg/kg/day in the 90-day study. In this regard, the data support a NOEL of 60 mg/kg/day (HDT) for adverse effects on the optic system.

The 90-day rabbit study is considered of limited usefulness by TB for the evaluation of the potential for MTA to affect the optic system not only because of the high rates of deaths apparently due to "mucoid enteritis" but also because of the selection of the high dose test level. Since this study was designed to assess for potential effects on the optic system, a dose level which is known to produce optic tract lesions as noted in the 21-day study should have been selected as the highest test dose level.

c. Summary: Effects in Rabbits

In three of the four studies with rabbits reviewed here and in two previous studies (refer to J. Whalan review dated September 5, 1985), MTA treatment was associated with functional effects in the eye and optic nerve and/or optic chiasma lesions. In each of the three studies with rabbits reviewed here, the testing laboratory study report concludes that MTA treatment results in eye effects and optic nerve and/or chiasma lesions.

The possibility that the lesions in the optic tract and functional effects in the eye as being the result of an Encephalitozoon cuniculi infection in the three studies reviewed here was considered by TB but the available evidence does not provide a sufficient basis to conclude that this parasite alone was responsible for the apparent effects of MTA treatment. The reasons for this position are as follows:

1) None of the control rabbits in either of the three studies considered useful had the optic tract lesions or functional effects in the eye.

2) The rabbits obtained for the acute dermal toxicity study were obtained Specific Pathogen Free (SPF) with respect to the presence of Encephalitozoon cuniculi. The study report states that these rabbits were further tested for the presence of this pathogen and found to be free of its presence.

   [Note: Although the test may have been conducted and the rabbits found to be free of the parasite, no information was provided which indicated when the rabbits were tested. TB recognizes that the rabbits might have become infected after the test and before the rabbits were assessed for the effects of MTA.]

3) Rabbits infected with Encephalitozoon cuniculi also have associated kidney lesions. The acute dermal and subchronic toxicity (21-day) studies were examined for kidney lesions and there was no evidence of a prevalence of infection with this parasite. Note: The kidneys were not examined microscopically in the acute oral toxicity study.

4) Several of the rabbits in these studies were reported to have the Encephalitozoon cuniculi infection but this was evidenced by "multifocal lymphocytic infiltration" usually in the brain. There was no pattern of there being more animals infected in the rabbits dosed with MTA than in the controls.

5) None of the references checked by this reviewer indicated that rabbits infected with

Encephalitozoon cuniculi develop functional changes in the eye such as mydriasis, nystagmus, delayed or absent pupil response or delayed palpebral response.

6) According to Dr. Lynnard Slaughter (TB consulting pathologist) the structure of the histopathological lesions which were noted in MTA treated rabbits (chronic granulomatous inflammation, necrosis, etc.) are more consistent with a parasite infection than they are with known responses to chemical insults. If the optic nerve and chiasma lesions are due to infection with Encephalitozoon cuniculi, then it is possible that treatment with MTA exacerbates the infection such that the infection becomes strong enough to result in the microscopic changes noted. If this is true, then MTA should still be regarded as toxic at the levels in which it may exacerbate the expression of the parasite. There was, however, no definite evidence of increased infection with Encephalitozoon cuniculi organisms in either the brain or kidney, the primary affected organs in MTA treated rabbits in either the acute or 21-day dermal studies.

   The possibility that MTA can exacerbate parasitic and other infections is, however, again indicated by the many deaths noted in the rabbits in the 90-day study when compared with the control groups which had only a few deaths due to the "mucoid enteritis" condition.

   TB considers the possibility that MTA exacerbates pathogenic infections in rabbits as being speculative and no further experimentation is required to clarify this possibility at this time.

d. Other considerations with regard to the potential of MTA to affect the eye and optic nerve and/or chiasma of rabbits are as follows:

   1) Multiple dosing does not result in marked cumulative effects on the eye or lesions in the optic nerve and/or chiasma as indicated by the 21-day dermal toxicity study and also

indicated by the limited data available from the 90-day study.

2) The testing laboratories (both IRDC and the Chevron Environmental Health Center) made the assertion that MTA affects the eye and optic nerve and/or chiasma in their report conclusions even though they were aware that the Encephalitozoon cuniculi infection may cause similar lesions and they recognized the need to protect the rabbits from this parasite.

2. Lack of Effects of MTA in the Optic System of the Rat

Four studies with rats were presented (acute oral, dermal and inhalation and 21-day dermal toxicity studies). None of these studies demonstrated definite indications of effects of MTA on the optic system (including the eye, optic nerve and optic chiasma) or pituitary. The only possible effect noted on the ocular system was an occasional and transitory delayed pupillary response in a few of the treated rats.

Thus, TB concludes that, at the dose levels tested, which were near lethal doses, MTA does not affect the optic system in rats.

No further testing in this species is required.

Studies Reviewed

81-1. Acute Oral Toxicity - Rats Chevron Environmental Health Center, #CEHC 2743 and Chevron #S-2993, December 21, 1987. [MRID No. 405048-33]	LD_{50}s = 426 (349-523) mg/kg males 519 (420-750) mg/kg females Toxicity Category III CORE GUIDELINE
81-1. Acute Oral Toxicity - Rabbits Chevron Environmental Health Center, #CEHC 2744 and Chevron #S-2994, December 22, 1987. [MRID No. 405048-34]	LD_{50} = 261 (197-406) mg/kg both sexes. Lesions in optic tract noted. CORE SUPPLEMENTARY
81-2. Acute Dermal Toxicity - Rats Chevron Environmental Health Center, #CEHC 2779 and Chevron #S-3048, December 21, 1987 [MRID No. 405048-36]	LD_{50}s = 1.92 (1.55-2.39) mg/kg males 1.41 (1.14-1.76) mg/kg females No optic lesions noted. CORE GUIDELINE
81-2. Acute Dermal Toxicity - Rabbits Chevron Environmental Health Center, #CEHC 2644 and Chevron November 20, 1987. [MRID No. 405048-35]	LD_{50} not established. 7/20 and 2/20 rabbits died at 250 and 175 mg/kg #S-3053, respetively. Toxicity Category either I or II. Study demonstrated MTA causes optic tract lesion following single dermal doses of 175 or 250 mg/kg. CORE SUPPLEMENTARY
81-3. Acute Inhalation Toxicity - Rats Chevron Environmental Health Center, #CEHC 2733 and Chevron #S-3006, May 5, 1987. [MRID No. 405048-37]	No deaths after 4 hours exposure to 2.8 mg/L. No evidence of pathological lesions in optic tract. CORE MINIMUM
82-2. 21-Day Dermal Toxicity - Rats Chevron Environmental Health Center, #CEHC 2734, and Chevron #S-3007, December 14, 1987. [MRID No. 405048-38]	NOEL < 50 mg/kg/day. At this this level there are local dermal reactions at the site of application and salivation. At 100 mg/kg/day there are decreases in

	platelet and RBCs. Severe dermal reactions result at 500 and 850 mg/kg/day. CORE GUIDELINE
82-2. 21-Day Dermal Toxicity - Rabbits IRDC, #415-047 and Chevron #S-3008, December 30, 1987. [MRID No. 405048-39]	NOEL < 5 mg/kg/day for local effects at site of application. NOEL = 50 mg/kg/day (systemic) LEL = 100 mg/kg/day lesions in the optic nerve and optic chiasma and one death. Delayed pupillary response. Deaths and moribund condition at 200 mg/kg/day and above. CORE GUIDELINE.
82-3. 90-Day Dermal Toxicity - Rabbits IRDC, #415-048, #CEHC 2822 and Chevron # S-3057, January 15, 1988. [MRID No. 405048-40]	Study is classified as INVALID. The rabbits in <u>all</u> groups showed "mucoid genteritis" but the rabbits in the groups dosed with MTA had higher rates of death by this infection. A possible effect of MTA treatment on increased death could not be firmly established but was strongly suggested. None Of the surviving treated rabbits developed lesions In the optic nerve or optic chiasma (NOEL > 60 mg/kg/day). INVALID
84-2. Mutagenicity (Ames Test) Microbiological Associates #T5771.501014 and Chevron #S-3108, December 23, 1987. [MRID No. 405048-41]	Not demonstrated to be mutagenic in strains TA98, TA100, TA1535, TA1537 or TA1538 with or without S-9 metabolic activation at dose levels up to and including 10,000 ug/plate. ACCEPTABLE

84-2. Genetic Toxicity (Rat Bone Marrow Cytogenetic Assay) Hazleton, #2107-147 and Chevron #S-2891, November 12, 1987. [MRID No. 405048-42]	No structural chromosomal aberrations at dose levels up to and including 2.21 mg/L (four exposures) dose levels which cause 20% decrease in body weight. UNACCEPTABLE
84-2. Genetic Toxicity (Micronucleus Assay in Bone Marrow Erythrocytes), Chevron Environmental Health Center, #CEHC 2751 and Chevron #S-2996, January 15, 1988. [MRID No. 405048-43]	No structural chromosomal aberrations at dose levels up to and including 3.00 mg/L following 4 hours inhalation. ACCEPTABLE

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/5/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 81-1 - Acute Oral Toxicity - Rats

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-33

Test Material: Methylthioacetate (SX-1763, 99.2% purity)

Synonyms: MTA

Study Number(s): S-2993 and CEHC 2743

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center, Inc.

Title of Report: The Acute Oral Toxicity of Methylthioacetate (SX-1763) in Adult Male and Female Rats.

Author(s): K.K. Dougherty, Ph.D., D.A.B.T.

Report Issued: December 21, 1987

Conclusions:

LD_{50} = 426 (349 to 523) mg/kg for males
= 519 (420 to 750) mg/kg for females
Toxicity Category II. No lesions in optic tract noted.

Classification: Core-GUIDELINE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling on December 30, 1987 indicating that two reviews (one on December 29, 1987 and the other on December 30, 1987) were made. No inspections during the in-life phase were made since the study report is dated December 21, 1987.

REVIEW

In this study five groups of 15 male and 15 female rats (Sprague-Dawley Crl:CD®BR, 62 to 69 days old) were dosed with either 0, 130, 230, 420, or 750 mg/kg of the test material (MTA, methylthioacetate) in peanut oil. The rats were fasted prior to dosing which was via intragastric intubation. Following dosing, the rats were observed for reactions up to 14 days. On days 3, 6, and 14, five rats of each sex were sacrificed by CO_2 asphyxiation and necropsied.

Results:

The rats which died as a result of MTA treatment died on day 0 (the day of dosing). Fourteen high-dose (750 mg/kg), seven in the second highest dose (420 mg/kg), and one in the next lower dose (230 mg/kg) group of males died. Among the females, all of the high-dose and two of the group receiving 420 mg/kg died as a result of treatment. The symptoms which preceded death included convulsions, fasciculation, paddling movement with forelimbs, no movement, and hunched posture. Signs which were noted in rats that survived included collapse, decreased motor activity, salivation, dyspnea, red or colorless nasal discharge, and increased respiration rate, as well as lacrimation/colorless eye discharge, ataxia, and diarrhea. Body weights were not affected. Most of the symptoms persisted for only an hour or so.

The following LD_{50}s were determined:

426 (349 to 523) mg/kg for males
519 (420 to 750) mg/kg for females

Necropsy of the rats which died as a result of MTA treatment revealed mottled brown or dark lungs; foamy material in the trachea, red, beige/tan, dark, and mottled livers; sloughing of the gastric mucosa; and red or a yellow contents in the small intestines. No effects on the optic tract were reported. Necropsy of the survivors was unremarkable.

Special histological assessment of the brain (including the medulla oblongata, pons, cranial nerves and optic chiasma), eyes with optic nerve attached, pituitary, and a section of the liver was made for the rats scheduled for sacrifice at day 14 for the control group and the rats dosed with 420 mg/kg and for the single rat surviving 750 mg/kg. The tissues were collected at necropsy and fixed. They were then sent to the Histo-pathology Laboratory for further preparation. Dr. Ward R. Richter who was responsible for preparing the pathology report and presumably the pathologist who examined the slides determined that the brain, eye, optic nerve and pituitary were "within normal limits."

TB notes that only lesions in the optic system that would have persisted to day 14 would have been detected microscopically since no tissues were microscopically examined from the rats sacrificed at days 3 and 6. It is possible that the rat optic system could have been affected by MTA treatment but the effects were transient. This possibility is evaluated better in the 21-day dermal toxicity study.

Conclusion:

This study is CORE-GUIDELINE. Sufficient data have been generated to classify technical MTA as Toxicity Category II. No effects of MTA treatment on the ocular system were noted in this study.

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/5/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 81-1. Acute Oral Toxicity - Rabbits

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-34

Test Material: Methylthioacetate (SX-1763, 99.2% purity)

Synonyms: MTA

Study Number(s): S-2994 and CEHC 2744

Sponsor: Chevron Chemical Company, Inc.

Testing Facility: Chevron Environmental Health Center, Inc.

Title of Report: The Acute Oral Toxicity of Methylthioacetate (SX-1763) in Adult Male and Female Rabbits.

Author(s): K.K. Dougherty, Ph.D., D.A.B.T.

Report Issued: December 22, 1987

Conclusions:

LD_{50} = 261 (197 to 406) mg/kg for both sexes.

This study provides evidence that MTA affects the optic tract (optic nerve and optic chiasma) by causing inflammation following a single oral dose of either 150 or 250 mg/kg of MTA. Mydriasis, nystagmus and absent pupil response were also evident as clinical signs in the treated rabbits.

Classification: Core-SUPPLEMENTARY

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A quality assurance statement signed by B.M. Dowling attested that four reviews were made: two during the in-life phase and two of the raw data and draft of the final report.

REVIEW

In this study, five groups of rabbits (New Zealand White, 10 to 15 weeks old when dosed) were fasted and dosed with either peanut oil alone or peanut oil containing 50, 150, 250 or 400 mg/kg of methylthioacetate (MTA). The control group and the group receiv-ing 150 mg/kg consisted of 10 rabbits per sex. The other groups consisted of 5 rabbits per sex. The rabbits were dosed intragastrically at the rate of 2 mL/kg of body weight. The extra rabbits in the control group and the group receiving 150 mg/kg were sacrificed on day 6 for special examination for possible lesion development, in particular in the optic nerve and optic chiasma. The remaining rabbits were sacrificed on day 14. Some of the rabbits from the original groups were replaced when it appeared that they had leg or back injuries. The sacrificed rabbits were subjected to gross necropsy and selected tissues were further evaluated histologically. The rabbits which died on the day of dosing were not examined histologically. The brain, eyes with optic nerve attached, pituitary, liver, and abnormal tissues were preserved and saved for histopathological analysis. Ultimately, the tissues from the rabbits dosed with 0, 150 and 250 mg/kg were assessed microscopically. A separate pathology report was prepared by Dr. Ward A. Richter.

Results:

An LD_{50} of 261 (197 to 406) mg/kg for males and females was determined. The deaths which occurred were within 13 minutes to 1 day following dosing. The symptoms included reduced food consumption, collapse, cyanosis, dyspnea, increased respiratory rate, salivation, ataxia, decreased motor activity, and mydriasis (extreme dilation of the pupil). At higher dose levels there were convulsions, nystagmus (involuntary rapid movement of the eyeball), and absent pupil response which persisted to 14 days in 2 of 5 rabbits. Other generalized symptoms were also noted. There was no effect on body weight gain. Most of the symptoms were transient and persisted for an hour or so except for the absent pupil response. The following table (see next page) illustrates the changes in the functional parameters of the eyes. It is readily apparent that only the eyes of the rabbits dosed with MTA are affected.

Dose Level mg/kg)	Effect: Mydriasis[1] M	Mydriasis[1] F	Nystagmus[2] M	Nystagmus[2] F	Pupil Response[3] M	Pupil Response[3] F
0	0	0	0	0	0	0
50	0	0	0	0	0	0
150	1	0	0	0	0	0
250	4	3	1	0	2	2
400*	5	2	3	0	0	1

Note: 5 rabbits per sex per group except for 10 rabbits per sex per group for the control and 150 mg/kg dose groups.
*5 of 5 rabbits died as a result MTA treatment
[1]Mydriasis was reported present from 5 minutes post dosing to day 1.
[2]Nystagmus was reported present from 3 minutes to 1.5 hours.
[3]Delayed or absent pupil response was reported present from day 1 to day 14.

Necropsy of the rabbits dosed with MTA revealed mottled or red spotted lungs, dark spleen, red foam in the trachea, and tan, white, and black grey areas in the liver.

A total of 47 rabbits from the controls and the dosed groups were examined microscopically. Of these 8 of the 27 rabbits in the groups dosed with MTA but none of the control rabbits had lesions in the optic nerve of optic chiasma as follows:

Males: 150 mg/kg

Nine rabbits were examined and seven were considered normal. One rabbit had "gliosis" of the optic nerve, another had vacuolar change and necrosis in the optic nerve. Both of these were sacrificed at day 6. Two rabbits had chronic granulomatous inflammation (CGI) of the brain and these same rabbits were considered to show evidence of an encephalitozoon infection.

Females: 150 mg/kg

Ten rabbits were examined and seven were considered normal. Two rabbits had CGI in the optic nerve and both were in the 14 day sacrifice group. The third affected rabbit had infiltration of the optic nerve which was considered as evidence of an encepalitozoon infection.

Males: 250 mg/kg

Four rabbits were examined and two were considered normal. Two rabbits had CGI in either the optic nerve or optic chiasma. A third rabbit had CGI of the brain which was considered evidence of an encephalitozoon infection.

Females: 250 mg/kg

Four rabbits were examined and three were considered normal. One rabbit had CGI in both the optic nerve and the optic chiasma.

The above information suggests that MTA affects the optic nerve (causes chronic granulomatous inflammation and possibly vacuolar change, gliosis and/or necrosis) and optic chiasma (inflammation). These lesions plus the observation of mydriasis, nystagmus and absent pupil response as symptoms in the treated rabbits further suggests that MTA has the potential to affect the rabbit optic system. None of either the functional or microscopic effects were reported to be present in the control rabbits. Although some rabbits had evidence of the encephalitozoon infection, not all rabbits showing optic tract lesions were infected.

Conclusion:

This study is CORE SUPPLEMENTARY. An LD_{50} of 261 (197 to 406) mg/kg is established (Toxicity Category II) for rabbits. The study suggests that MTA affects the optic tract of rabbits as indicated by inflammation of the optic nerve and optic chiasma, vacuolar change and/or necrosis of the optic nerve and functionally by causing mydriasis, nystagmus and absent pupillary response.

Note: The study report conclusion concedes that MTA treatment affects the optic tract. For example, the following is a quote from the study summary (page 3) "The test material caused optic tract lesions at 150 and 250 mg/kg in both males and

females. The no-effect level for optic tract lesions was 50 mg/kg."

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/5/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 81-2 - Acute Dermal Toxicity - Rats

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-36

Test Material: Methylthioacetate (SX-1763, 99.2% purity)

Synonyms: MTA

Study Number(s): S-3048 and CEHC 2779.

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center

Title of Report: The Acute Dermal Toxicity of Methylthioacetate (MTA) (SX-1763) in Adult Male and Female Rats.

Author(s): K.K. Dougherty, Ph.D., D.A.B.T.

Report Issued: December 21, 1987

Conclusions:

LD_{50} = 1.92 (1.55 TO 2.39) g/kg for males
LD_{50} = 1.41 (1.14 to 1.76) g/kg for females
Persistent local dermal irritation results. Toxicity Category II.

Classification: Core-GUIDELINE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling attesting that a review of the raw data and draft final report was provided. No in-life inspections or protocol inspections were reported as being made.

REVIEW

Four groups of 15 rats of each sex (Sprague-Dawley Crl: CD®BR, 71 to 78 days of age) were dosed with a single dose of either 4.0, 2.0, 1.0, or 0.5 g/kg dermally. The test material (MTA) was kept in contact for 24 hours by means of a plastic sheet and bandage. Five rats from each group (if available) were sacrificed by CO_2 asphyxiation on day 3, 6, or 14 after dosing to assess for the onset of or for early lesions.

Results:

LD_{50}s as follows were determined:

1.92 (1.55 to 2.39) mg/kg for males
1.41 (1.14 to 1.76) mg/kg for females

All of the rats dosed with 4.0 mg/kg died and all deaths occurred on the day of dosing. The symptoms of toxicity included ataxia, absence of movement, dyspnea, salivation, collapse, fasciculation, and tremors as well as red/foamy discharge (nasal), decreased motor activity, and red/colorless discharge. The symptoms abated after day 1.

The test material produced severe dermal irritation which persisted to 14 days and which was evident at the site of application. No PIS score was determined. No effects on body weight gain were evident.

Aside from local dermal reactions, there were no dose-related macroscopic lesions in the internal organs. The eyes and optic tract were reported as being normal.

All rats in the group dosed with 0, 0.5, 1.0 and 2.0 mg/kg and five rats of each sex in the group dosed with 4.0 mg/kg of MTA were necropsied. The necropsy included the brain (with optic chiasma), eyes (with optic nerve attached). Microscopy of the brain (including the medulla oblongata, pons, cranial nerves and optic chiasma), eyes and optic nerves as well as other organs was done on the rats dosed with 0, 1.0 and 2.0 mg/kg of MTA. Since the rats dosed with 4.0 mg/kg died on day 1 they were not assessed microscopically. The tissues were taken at necropsy and prepared for microscopy at the Histopathology Laboratory. According to Dr. Ward R. Richter who prepared the pathology report, there were no effects of MTA in either the brain, optic nerve or eye.

Conclusion:

This study is CORE GUIDELINE.

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/2/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 81-2 - Acute Dermal Toxicity - Rabbits

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-35

Test Material: Methylthioacetate (SX-1724, 99.4% purity)

Synonyms: MTA

Study Number(s): S-3053 and CEHC 2644.

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center

Title of Report: The Acute Dermal Toxicity of Methylthioacetate (MTA) (SX-1724) in Specific Pathogen-Free Rabbits.

Author(s): J.R. Cushman, Ph.D., D.A.B.T.

Report Issued: November 20, 1987

Conclusions:

LD_{50} not established. MTA may be Toxicity Category I or II since there were 7/20 deaths at 250 mg/kg and 2/20 deaths at 175 mg/kg. The study provides evidence that MTA affects the optic tract following a single dermal dose of either 175 or 250 mg/kg.

Classification: Core-SUPPLEMENTARY

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling attested that three reviews were made of the raw data and the draft final report. No in-life inspections were made.

REVIEW

In this study, three groups of 10 rabbits of each sex (New Zealand White Hra:(NZW)SPF) which were raised specific pathogen free (SPF) with respect to the organism Encephalitozoon cuniculi were dosed with a single dose of MTA at either 175 or 250 mg/kg (undiluted). The third group served as the untreated control. The purpose of using the SPF rabbits was because MTA was, in other studies, shown to cause optic nerve lesions that possibly resemble in morphology lesions produced in the central nervous system by Encephalitozoon cuniculi. Prior to dosing, the rabbits were clipped and the test material kept in place by means of a plastic sheet. Contact was for 24 hours. One set of rabbits (5 per sex per group) was treated and sacrificed after 14 days. Another set was treated and sacrificed after 3 days in order to detect any early potentially transient effect in the optic or visual system.

Results:

MTA treatment was fatal to two (one male and one female) rabbits dosed with 175 mg/kg. At 250 mg/kg, three males and four females died. Deaths (except one) occurred within 72 minutes of dosing. The symptoms preceding death included collapse, dyspnea, decreased respiration, absent palpebral response, mydriasis, cyanosis, salivation, and decreased motor activity and in some cases ataxia, decreased body temperature, and absent pupil response. Absent pupil response was also noted in some of the survivors on day 1. The study report states that the increased mortality between the first group of rabbits dosed (6/10) and the second (1/10) dosed with 250 mg/kg may have been due to the difference in air flow in the caging. The air flow was increased for the second dosing period to decrease the odor. The following table illustrates the functional reactions in the eyes of the rabbits in this study.

Dose Level (mg/kg/day)	Mydriasis[1]		Pupil Response[2]		Palpebral Response[3]	
	M	F	M	F	M	F
0	0	0	0	0	0	0
175	4	5	4	5	6	4
250	7	3	4	3	7	1

Note: 10 rabbits per group.
[1]Mydriasis was reported starting at about 17 min and lasting up to 14 days.
[2]Absent or delayed pupil response was reported starting at about day 1 and lasting to day 14.
[3]Delayed palpebral response was reported on day 1 only.

A primary skin irritation score was not determined but the report describes the condition of the skin of the rabbits dosed with MTA. Since the skin showed effects of MTA persisting to day 14, MTA is a severe irritant. No effects on body weight were evident. Blood samples were negative for the presence of E. cuniculi infection but the actual results of the assays were not presented.

Ophthalmological examination consisted of measuring the pupillary movement to direct light assessed prior to mydriases. The eyes were examined with a Swiss hand-held slit lamp biomicroscope and a Xonax indirect ophthalmoscope. The results of ophthalmoscopic examination were presented in a series of tables consisting of 18 pages (Appendix A of the study report). The results of the ophthalmoscopic examination are shown in the following table taken from the study report.

> "The incidence of absent or incomplete pupil response immediately before scheduled sacrifice is presented below; most of these animals had no other ophthalmological abnormalities. The absent or incomplete pupil responses are considered to be related to treatment with MTA."

Incidence of
Absent or Incomplete
Pupil Response

Dose (mg/kg)	Sex	Day 3 Sacrifice	Day 14 Sacrifice
0	Male	0/5*	0/5
	Female	0/5	0/5
175	Male	0/4	1/5
	Female	3/5	1/4
250	Male	3/5	0/2
	Female	2/4	1/2

*Incidences/number of rabbits examined.

Since none of the control rabbits had evidence of affected pupil response at either day 3 or day 14, these data provide evidence that MTA affects the pupil response and this effect persists to day 14 in some of the treated rabbits.

Gross necropsy revealed several lesions in the rabbits which died as a result of MTA treatment. These included abraded or red skin, firm blanched liver with brown tips, red and mottled lungs, enlarged and discolored thymuses, a dark or red kidney, and other findings. The cause of death was not described except for one rabbit which the report states may have died of liver necrosis.

The results of the histopathological examination of the optic nerve revealed several lesion types which included necrosis, chronic granulomatous inflammation, necrotic inflammation, vacuolar change, and myelin degeneration as indicated in the following information taken from the pathologist's report for this study. (Data are from all of the rabbits from both interim and terminal sacrifices and deaths when available.)

	Group I Controls		Group II 175 mg/kg		Group III 250 mg/kg	
	M	F	M	F	M	F
Optic Nerve	(10)*	(10)	(10)	(9)	(7)	Eye, (6)
Degeneration, Myelin	0	0	1	0	0	0
Inflammation, Chronic Granulomatous	0	0	4	2	1	2
Inflammation, Necrotic	0	0	1	0	0	0
Necrosis	0	0	1	3	5	2
Vacuolar Change	0	0	1	1	1	0
Within Normal Limits	10	10	4	4	0	2

*Number of rabbits examined.

Of these lesions the most evident possible effects of MTA treatment are revealed as chronic granulomatous inflammation and necrosis. Nine of the rabbits were reported as having evidence of an encephalitozoon infection.

The optic chiasma was not specifically listed in the pathology table but the text of the report (page 10 of the text) states that these lesion types (as above) were present in this structure.

Liver lesions consisting of centrilobular necrosis and vacuolar change were also described histologically in the rabbits sacrificed at day 3 but not at day "4" (apparently a reference to day 14).

Although SPF rabbits were used, an encephalitozoon infection may have developed at the testing laboratory to cause the lesions.

There was, however, no evidence of dose related increases in the incidences of lesions in the kidney that would indicate that treatment with MTA exacerbated the proliferation of encephalitozoon infections.

Conclusion:

This study is SUPPLEMENTARY. The LD_{50} was not determined. Due to high incidences of deaths (7/20 at 250 mg/kg), the test substance may require classification as Toxicity Category I. The study presents very important observations regarding the dermal toxicity of MTA. For example, it demonstrates that MTA is toxic via this route (either Category I or II) and causes severe skin irritation.

The study also demonstrated that MTA may also affect the optic nerve following a single dermal exposure as indicated by only the rabbits dosed with MTA developing histopathological changes (chronic granulomatous inflammation, vacuolar change, necrotic inflammation and myelin degeneration). Functional changes (mydriasis, and absent and/or delayed pupil and palpebral responses) were also noted only in the rabbits dosed with MTA.

Note: The testing laboratory conceded that MTA treatment affects the optic tract. For example, the following quotation is from the study summary report "A single dose of 175 or 250 mg/kg in Encephalitozoon cuniculi free rabbits caused necrosis and myelin degeneration of the optic nerve, which was not reversible by day 14."

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/6/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 81-3 - Acute Inhalation Toxicity - Rats

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-37

Test Material: Methylthioacetate (SX-1732, 98.3% purity)

Synonyms: MTA

Study Number(s): S-3006 and CEHC 2733.

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center

Title of Report: The Acute Inhalation Toxicity of Methylthio acetate (SX-1732) in Rats.

Author(s): E.B. Bruce, B.S./L.C. Griffis, Ph.D.

Report Issued: May 5, 1987

Conclusions:

No deaths following 4 hours (240 minutes) exposure to methylthioacetate at 2.8 mg/L. No evidence of pathological lesions in the optic tract. Toxicity Category III.

Classification: Core-MINIMUM

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling attesting that two reviews of the raw data and/or draft report were made was provided. No in-life or protocol inspections were reported. A supplementary statement was also provided which concerned final report revisions.

REVIEW

Two groups of five rats of each sex (Sprague-Dawley rats, 56 days of age at the time of exposure) were used in this study. One group was exposed to filtered air only and served as the controls. The second group was exposed to an atmosphere containing 764 (± 127) ppm or 2.8 mg/kg of methylthioacetate (MTA) for 4 hours. The test atmosphere was generated by passing filtered air through a series of glass washing bottles, the first containing 175 mL and the second containing 100 mL of test material. The air flow was passed at the rate of 1.6 L/min to generate an air stream "saturated" with MTA vapors. The saturated air was delivered to the chamber supply air stream near the inlet at the top of the chamber and the diluted test vapor was forced into the chamber. The concentration of MTA in the chamber atmosphere was continuously monitored with a Miran-IA infrared gas analyzer. The rats were sacrificed 14 days following the exposure with an intraperitoneal injection of sodium pentobarbital.

Following sacrifice the rats were necropsied and the lungs, livers, kidneys, tracheas, eyes, optic nerves, brains (with optic chiasma) and pituitaries were prepared and evaluated histologically.

Results:

None of the rats died as a result of exposure. The symptoms of intoxication included salivation, squinted or closed eyes, decreased motor activity, labored breathing, unconscious or not moving (mainly during a period when the chamber atmosphere was approximately 1200 ppm), clear ocular discharge, diarrhea or yellow anogenital discharge, mydriasis (1 female). The rats recovered in from 40 minutes to 1 day. There was an initial body weight loss evident on day 2 but not afterwards.

There were no compound-related macroscopic or histopathological lesions related to the exposure to MTA reported. In particular, according to Dr. Ward R. Richter who prepared the separate pathology report, the eyes and optic nerve were reported as being normal.

Conclusion:

This study is CORE MINIMUM. Only a single group was exposed to the MTA and the atmosphere level was less than the limit test (5 mg/L). Sufficient data were generated to determine that MTA can be classified as Toxicity Category III. No effects on the optic tract were evident.

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/2/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 82-2 - 21-Day Dermal - Rats TOX Chem No.: 584D 2A

Accession Number: MRID No.: 405048-38

Test Material: Methylthioacetate (SX-1732, 98.3% purity)

Synonyms: MTA

Study Number(s): S-3007 and CEHC 2734

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center

Title of Report: Three-Week Repeated-Dose Dermal Toxicity Study in Rats with Methylthioacetate (SX-1732).

Author(s): K.K. Dougherty, Ph.D., D.A.B.T.

Report Issued: December 14, 1987

Conclusions:

NOEL < 50 mg/kg/day. At this level there are local dermal reactions at the site of application and salivation. At 100 mg/kg/day there are decreases in platelet and red blood cells. Severe dermal reactions result at 500 and 850 mg/kg/day. Levels tested: 0, 50 and 100 mg/kg/day for 21 days; the test groups receiving 500 and 850 mg/kg/day were terminated. There were no effects evident in the brain, eye or optic nerve.

Classification: Core-GUIDELINE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling attesting that reviews were made on two occasions on the raw data and draft of the final

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report. No in-life reviews or inspections were reported as being made.

REVIEW

The basic design of this study consisted of five groups of five male and five female rats (Sprague-Dawley® Crl:CD®BR, 62 to 71 days of age at initiation of dosing) which were dosed by dermal application with either 0, 50, 100, 500, or 850 mg/kg/day of MTA for 16 doses over a period of 21 days. The test material was applied undiluted and kept in contact with the skin for 6 hours for each application by means of a plastic film and tape.

Results:

1. Mortality - The rats in the groups receiving 500 and 850 mg/kg/day were discontinued from the study after 1 and 5 days of application, respectively, because one rat in the group receiving 850 mg/kg died and the others developed such severe skin reactions (see below) sacrificing the rats was appropriate.

2. Signs of Toxicity - The symptoms of toxicity noted included "collapse" (in the 500 and 850 mg/kg groups); the group receiving 100 mg/kg and above demonstrated decreased motor activity, phonation, increased respiratory rate and labored breathing. Salivation was reported in all dosed groups. Slow pupil response was noted in a few treated rats at day 0 but was considered normal thereafter.

   NOEL < 50 mg/kg/day for behavioral reactions.

3. Dermal Reactions - Test material related dermal reactions were reported at all dose levels. The rats dosed with 50 mg/kg exhibited dry and/or flaky skin, scabbing, swollen, sloughing, and/or scarred skin. Necrotic and thickened skin was observed at 100 and 500 mg/kg. Females were reported to have been more severely affected than males. On day 21, "slight to severe erythema with well-defined to moderate edema" was observed in females dosed with 50 mg/kg but the males in this dosage group were reported to be similar to the controls.

   NOEL < 50 mg/kg/day for dermal (site of application) effects.

4. Body Weight and Food Consumption - No compound-related effects were reported on body weight, body weight gain, food consumption, or relative food consumption.

[Note: For sections 5 and 6 below, blood samples were taken at the end of the study via the abdominal aorta.]

5. Hematology - The following parameters were investigated: white blood cell count, red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration.

   Of these parameters, red blood cell counts were reported as being depressed (-8%, $p \leq 0.05$) and platelet counts (-28%, $p \leq 0.05$) for females and the study report asserts that these decreases were probably related to the test chemical. Other decreases noted were not of significant magnitude or consistency to be considered effects of the test chemical by the testing laboratory.

   NOEL = 50 mg/kg/day

6. Clinical Chemistry - The following parameters were investigated by means of a Boehringer-Mannheim Diagnostics Group 8600R: Na^+, K^+, Cl^-, Ca^{++}, phosphorous, uric acid, BUN, creatinine, direct and total bilirubin, glucose (fasting), albumin/globulin ratio, indirect bilirubin, LDH, AST, ALT, CPK, cholesterol, triglycerides, total protein, alkaline phosphatase, albumin, globulin, and BUN/creatinine ratio.

   None of these parameters demonstrated a dose-dependent or compound-related effect of MTA treatment.

   NOEL = 100 mg/kg/day

7. Organ Weights - The liver, kidneys, gonads, and adrenals were weighed and their absolute and relative body weights were compared to controls.

   No dose-dependent or compound-related effect of MTA treatment was evident.

   NOEL = 100 mg/kg/day

8. Gross Necropsy - A necropsy was performed on all rats in the control, 50, and 100 mg/kg/day dosage groups and for the rats which died but these tissues and the tissues from the rats discontinued from the study were discarded. The protocol limited the gross necropsy investigation to some 19 tissues, including the brain with the eyes and optic nerve.

No compound-related lesions were recognized by the study report except for dermal reactions at the site of application.

9. Histopathology - The organs subjected to examination included the lungs, spleen, liver*, brain (with eyes and optic nerve attached)*, skin (treated and untreated areas), kidneys*, adrenals, testes, pituitary, ovaries, tissues with gross lesions*, sciatic nerve, and spinal cord. The "*" items were investigated for the 50 mg/kg/day group. All other tissues including the items with the * were investigated for the control and 100 mg/kg/day group.

   No compound-related lesions were recognized in the study report except for skin lesions. The pathology report (Appendix I) was prepared by Dr. Ward R. Richter. The skin lesions consisted of acanthosis and escharotic exudate.

   Toxicology Branch notes that there were no lesions reported in either the brain, eye, or optic nerve. This observation resulting from a 21-day dermal toxicity study strongly supports the contention that MTA does not affect the ocular system of the rat. The acute oral and dermal toxicity studies with this species each suffered from the shortcoming that since the optic system was not assessed microscopically until after 14 days, transient effects on the ocular system may have been missed. Since in this study, the histology was assessed within a short period after the last dose, the possibility of missing transient effects of MTA was minimized. It is unlikely that an early transient (within the first few days of dosing) effect would have reversed itself during the course of the daily treatment with MTA.

Conclusion:

This study is CORE GUIDELINE for a 21-day repeated dose dermal toxicity study. The study did not demonstrate a NOEL for dermal (site of test material application) effects or for clinical signs (salivation). Other toxic signs include decreases in platelet and red blood cell counts occurring at 100 mg/kg/day. It is recognized that severe reactions to the test chemical occur at 500 mg/kg/day and above.

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/5/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 82-2 - 21-Day Dermal - Rabbits TOX Chem No.: 584D 2A

Accession Number: MRID No.: 405048-39

Test Material: Methylthioacetate (MTA)

Synonyms: MTA

Study Number(s): Chevron #S-3008, IRDC #415-047 and CEHC 2823

Sponsor: Chevron Chemical Company

Testing Facility: International Research and Development Corp. (IRDC)

Title of Report: Twenty-One Day Dermal Toxicity Study in Rabbits with Methylthioacetate (MTA).

Author(s): J.R. Cushman, Ph.D., D.A.B.T.

Report Issued: December 30, 1987

Conclusions:

NOEL < 5 mg/kg/day for local irritation effects at site of application.
NOEL = 50 mg/kg/day for systemic effects.
LEL = 100 mg/kg/day, lesions in optic nerve and optic chiasma, more definite delayed pupil response. One death. Deaths or moribund condition resulted at 150 mg/kg and above.

Classification: Core-GUIDELINE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling attesting that two reviews were made, one of the pathology raw data and draft final report, and one of the draft final report. No in-life inspections or reviews were indicated as being made. A Quality Assurance statement was also provided by the IRDC laboratory and signed by Margery J. Wirth attesting that 14 inspections were made between May 18 and October 13, 1987, apparently covering the protocol to the final report.

REVIEW

In this study the basic study design consisted of five groups of five male and five female rabbits (New Zealand White, obtained from Hazleton Dutchland, Denver, PA; approximately 2 months old) were dosed with either 0, 5, 50, 100, or 200 mg/kg of methylthioacetate (MTA, SX-1732). The test material was administered undiluted in the area between the shoulder and the rump, which had been clipped free of hair. It was spread about this area with a glass rod and bandaged with plastic wrap and secured with Dermiform tape. The test material was kept in place for "approximately 6 hours" before removal and the area blotted. The dose levels were based on a preliminary (4-day) study which indicated that the rabbits should tolerate 250 mg/kg/day. The original dose levels for the two highest dose groups were 300 and 150 mg/kg but these were reduced to 200 and 100 by day 2 or day 3 because of deaths and toxic reactions to treatment. Dosing was for 5 days per week for 21 days.

Results:

1. Mortality - The following rabbits died or were sacrificed:

   - o A female dosed with 300 mg/kg on day 1;
   - o Two males dosed with 150 mg/kg on day 2;
   - o Two females dosed with 250 mg/kg on day 2;
   - o Two control females (on days 6 and 10);*
   - o A female dosed with 100 mg/kg on day 9;
   - o Three males dosed with 200 mg/kg (between days 4 and 10); and
   - o All females dosed with 200 mg/kg (between days 4 and 10).

   (The remainder of the rabbits dosed with 200 mg/kg/day were sacrificed on day 11.)

   NOEL = 50 mg/kg/day for deaths. There was one death (a female) reported on day 9 in the group dosed with 100 mg/kg/day.

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   *These deaths were described as natural.

2. Clinical Signs and Behavioral Reactions - The only recognized test chemical-related reactions to treatment were reported as being prostration and labored breathing in the rabbits dosed with 100 and 200 mg/kg. These were present in 3 of the 10 rabbits in the group receiving 100 mg/kg and 7 of the 7 rabbits in the group receiving 200 mg/kg (the other three in this group died.).

   The signs and symptoms in the rabbits immediately preceding death were not reported.

   NOEL = 50 mg/kg for behavioral reactions to the test material

3. Dermal Irritation - The rabbits dosed with 5 mg/kg/day were noted to have "slight degrees of edema and erythema on study days 17-21." Signs of dermal irritation were increased at higher levels of exposure to MTA. At 50 mg/kg/day the signs included, in addition to the signs at 5 mg/kg/day, dark discoloration (3 animals, days 11-14) and fissuring (one rabbit on days 17-18). At 100 and 200 mg/kg/day edema and eschar formation developed.

   NOEL < 5 mg/kg/day for local dermal reactions

4. Body Weights and Food Consumption - No statistically significant decreases in body weights or food consumption were reported.

5. Ophthalmoscopic Examination

   a. Papillary Response - Sluggish pupillary responses were noted in two rabbits on days 8 and/or 9, respectively, for the group receiving 50 mg/kg/day and four rabbits on days 8, 9 and/or 10 for the group receiving 100 mg/kg/day. Prior to sacrifice on day 11, all of the rabbits dosed with 200 mg/kg/day exhibited impaired pupillary function. In one of the high-dose group rabbits, impaired pupillary response was noted as early as day 2.

      NOEL = 5 mg/kg/day (but arbitrary, see part b below)

   b. Ophthalmoscopic Examination - This examination consisted of dilation of the eyes with 1 percent tropicamide solution and examination with a binocular indirect ophthalmoscope. The clarity

of the ocular media (precorneal tear film, cornea, aqueous humor, lens, and vitreous humor) and fundus reflex were evaluated. In addition, the examination included viewing the ocular adnexa and iris. The ophthalmoscopic evaluation was provided by Dr. Waldo F. Keller, a Consulting Veterinary Ophthalmologist. Dr. Keller's conclusions for the surviving rabbits which were dosed with 100 mg/kg/day or lower which were subjected to terminal (21st day) ophthalmological examinations were that "all observations noted were within the limits of variation commonly encountered in animals of this sex, age and strain."

NOEL = 100 mg/kg/day for ophthalmoscopic effects (when assessed at day 21).
[Note: The rabbits that were dosed with 200 mg/kg/day were examined by IRDC personnel and it was noted that "all animals were observed with sluggish or no pupillary response."]

6. Hematology - The following parameters were investigated: leukocyte count, erythrocyte count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, differential leukocyte count, and reticulocytes.

The study report maintains that no definite test chemical effects were evident as a result of MTA exposure. There was a "suggestion" of "regenerative anemia" in the group receiving 200 mg/kg/day but since this group was sacrificed early, there was no control group for comparison.

NOEL = 100 mg/kg/day

7. Clinical Biochemistry - The following parameters were investigated: Alkaline phosphatase, total bilirubin, aspartate aminotransferase, alanine aminotransferase, urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, triglycerides, and cholesterol.

This study report maintains that no definite test chemical effects were evident as a result of MTA exposure. Serum triglycerides were reported as apparently being elevated in the group receiving 200 mg/kg/day but there was no control group sacrificed at the same time for direct comparison.

NOEL = 100 mg/kg/day

8. Organ Weights - The following organs were weighed: adrenals (2), kidneys (2), liver, lung, ovary (2), and testis (2). Note: paired organs (2) were weighed separately to assess for a unilateral difference.

   No compound-related differences in organ weights resulted or were recognized as being related to exposure to the test material.

   NOEL = 100 mg/kg/day

9. Gross Necropsy - The rabbits were sacrificed by intravenous sodium pentobarbital followed by exsanguination. Macroscopic evaluations were made on all sacrificed rabbits. The brain, pituitary, eyes, and optic nerve were collected as a unit and preserved in situ in the skull with neutral buffeted formalin and sent to the sponsor for histological evaluation. Other organs were examined and processed for histopathology and stained with hematoxylin and eosin and sent to the sponsor for evaluation.

   Necropsy revealed lesions at the site of application of the test material only. These included desquamation, exfoliation, scabbing and/or thickening in the rabbits dosed with 50 mg/kg/day and above. Only mild desquamation was noted in 3 of 10 rabbits in the group dosed with 5 mg/kg/day.

   NOEL < 5 mg/kg/day for local irritation effects
   NOEL = 100 mg/kg/day for systemic effects

10. Histopathology - The histopathological report was prepared by Ward R. Richter, D.V.M. of the Chevron Environmental Health Center, Inc. and is presented in Appendix B of the study report.

    Dr. Richter reported that compound-related lesions were noted in the optic chiasma in the rabbits dosed with 100 and 200 mg/kg/day. The following table adapted from the histopathology tables of the study report illustrates these observations.

Group	Brain Optic Chiasma[1]		Optic Nerve Inflammation, Chronic Granulomatous		Necrosis	
	M	F	M	F	M	F
Control	0	0	0	0	0	0
50 mg/kg	0	0	0	0	0	0
100 mg/kg	0	2	0	2	0	0
200 mg/kg[2]	1	0	2	1	1	0

[1]Optic chiasma lesion described as inflammation, chronicgranulomatous (1), moderate (1), total = 2.
[2]All rabbits died prior to day 11 or were sacrificed on day 11.

Neuritis was also present in the optic nerve but there were two incidences in the control and the dosed group receiving 100 mg/kg/day.

The above table suggests that the group dosed with 100 and 200 mg/kg/day developed histological changes in the optic nerve and optic chiasma of the brain.

TB recognizes that the frequency of rabbits affected with any one of the lesions listed in the table above is 1 or 2 out of 4 or 5 examined.

The rabbits in this study were noted to have an encephalitozoon infection and the presence of chronic encephalitis in the controls and some dosed rabbits but this condition was, according to the study report, not indicated as compromising the study.

Conclusion:

This study is CORE GUIDELINE. The following one-liner applies:

NOEL < 5 mg/kg/day for local irritation effects at site of application.
NOEL = 50 mg/kg/day for systemic effects.
LEL = 100 mg/kg/day, lesions in optic nerve and optic chiasma, more definite delayed pupil response. One

death. Deaths or moribund condition resulted at 150 mg/kg and above.

Note: The two rabbits showing signs of delayed pupil response on 1 or 2 days only when dosed with 50 mg/kg/day is not considered by TB to be serious enough such that 50 mg/kg/day should be assigned as the LEL.

Note: The testing laboratory (page 3 of the report) concedes that MTA treatment results in optic tract lesions (at 100 mg/kg/day) and sluggish pupillary response (without lesions at 50 mg/kg/day) and with associated lesions at higher levels.

Reviewed By: J.D. Doherty John Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 6/6/89
Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 82-3 - 90-Day Dermal Toxicity - Rabbits

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-40

Test Material: Methylthioacetate (MTA)

Synonyms: MTA

Study Number(s): Chevron No. S-3057, IRDC No. 415-048, CEHC 2822

Sponsor: Chevron Chemical Company

Testing Facility: International Research and Development Corporation, IRDC

Title of Report: Ninety-Day Dermal Toxicity Study in Rabbits with Methylthioacetate (MTA).

Author(s): J.R. Cushman, Ph.D., D.A.B.T.

Report Issued: January 15, 1988

Conclusions:

The study is INVALID because animals of poor health were used. The high percentage of deaths in the rabbits dosed with MTA together with the presence of the infection "mucoid enteritis" indicate a possible relationship between the deaths and chemical treatment but this could not be firmly established by the available data. No effects on optic nerve among the surviving rabbits at dose levels up to and including 60 mg/kg/day (HDT). Dose levels tested 0, 5, 20 and 60 mg/kg/day.

Classification: Core-INVALID

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by Margery J. Wirth attesting that the study was subjected to periodic inspections by the IRDC Quality Assurance Department. A second statement signed by B.M. Dowling indicated that two reviews of the pathology raw data and/or draft report were made by the Chevron group.

REVIEW

This study was designed to assess for 90-day subchronic toxicity of methylthioacetate in the rabbit. The study was especially designed to assess for and establish a NOEL for lesions in the optic nerve and optic chiasma to which, based on acute and subacute toxicity studies, this species (rabbit) appears to be susceptible.

The basic design for this study consisted of dosing via dermal application of undiluted test material four groups of New Zealand White rabbits (2 months of age at receipt and obtained from ARI Breeding Laboratories, East Bridgewater, MA) with either 0, 5, 20, or 60 mg/kg/day. The control, mid-, and high-dose groups were originally assigned 15 males and 15 females. It was later discovered that one of the females in the high-dose group was a male, thus there were 16 males and 14 females in this test group. The low-dose group consisted of 10 males and 10 females. The purpose of there being 15 rabbits of each sex in some groups was because the original protocol called for maintaining five rabbits of each sex for a recovery phase. This aspect of the study was cancelled because of the high rate of mortality (see below).

The test material was applied to the clipped backs of the rabbits covering 5 to 10 percent of the "application site" and spread about with a glass rod. The test material was covered with plastic wrap which was taped in place and kept in place for 6 hours after which it was removed and the area blotted of excess material. In order to minimize dermal irritation, the test material was applied at different sites of the application area (between the shoulder and the rump) beginning on day 16.

Results:

1. Mortality and Clinical Signs - The following table indicates the number of deaths noted during the in-life phase of this study.

Deaths and Enteritis

	Males		Females	
	Deaths	Mocoid Enteritis*	Deaths	Mucoid Enteritis*
Control	1	6	1	10
5 mg/kg	5	8	5	5
20 mg/kg	9	7	7	10
60 mg/kg	7	10	8	5

(Note: 15 rabbits per sex per dose)

*Mucoid Enteritis plus goblet cell hyperplasia.

There were many more deaths in the rabbits dosed with MTA than in the control group, but there was no dose response evident over the dose range of 5 to 60 mg/kg/day. A variety of symptoms preceded deaths which included emaciated appearance, prostration, ataxia, decreased activity, tremors, decreased defecation/no stool, reddish fluid in refuse pen, diarrhea/mucoid diarrhea, cold to touch, labored breathing, inappetence, shallow breathing, and anogenital staining. Most deaths occurred during study days 20 to 52. The necropsy record indicated that the majority of the rabbits which died had "mucoid enteritis," a condition that is described as having mucoid material in the intestine. The histopathology report further elaborated on this condition and based on the microscopic analyses it was determined that there was no increase in the incidence of mucoid enteritis among treated animals when compared to the controls. There was an increase in the number of deaths from mucoid enteritis among treated animals when compared to the untreated controls but not among the rabbits treated with MTA. Other rabbits which did not succumb to the "mucoid enteritis" condition and lived to terminal sacrifice had "goblet cell hyperplasia of the colon." This condition was considered by the pathologist (Ward R. Richter) to be related to the "mucoid enteritis" condition. The study summary report prepared by the sponsor also suggests that "there may have been an indirect effect of compound treatment which exacerbated the disease leading to the increased deaths."

Toxicology Branch (TB) considers that the high rate of deaths of the rabbits in this study may be related to either the combination of MTA plus the enteritis

condition or possibly also to some other aspect of MTA treatment. The fact that the controls for both sexes have such few deaths together with the fact that there were a total of eight deaths of unknown cause among the treated rabbits strongly suggests that MTA may be affecting the rabbits at all dose levels.

TB has determined that this study is INVALID because animals of poor health were initiated in the study or an infection in the test animals developed after the start of the study such that the objectives of assessing the 90-day dermal toxicity potential of MTA was compromised.

TB recognizes that the condition "mucoid enteritis" is a common decease among rabbits and its cause may be related to "an irritant, a toxin, or a stress-induced secretory alteration in the intestinal mucosa" (J.E. Harkness and J.E. Wagner, The Biology and Medicine of Rabbits and Rodents, Lea and Febiger, 1977, page 94). TB also recognizes that this condition is preventable and controllable with good laboratory management.

2. Body Weight and Food Consumption - No consistent effects of MTA treatment were evident on body weight or food consumption.

3. Pupillary Response and Ophthalmoscopy - Pupillary response was evaluated once weekly and a special ophthalmoscopic examination was conducted monthly and at pretest (Dr. Waldo F. Keller was the veterinarian responsible for the ophthalmology evaluation report).

   No effects of MTA were reported as developing in the treated rabbits.

4. Dermal Irritation - Signs of local dermal reactions were reported as developing in all groups dosed with MTA. The group dosed with 5 mg/kg/day developed slight erythema and edema and a few developed desquamation. The higher dose levels of MTA treatment resulted in more severe dermal reactions.

5. Hematology and Clinical Biochemistry - No test chemical effects on any of the hematological parameters investigated were reported.

   The only clinical biochemistry which showed a possible effect of MTA treatment was an apparent dose-related decrease in lactic dehydrogenase. Decreases in the

levels of the activity of this enzyme are not recognized to have toxicological significance. Thus, no effects of MTA on clinical biochemistry parameters resulted in this study.

6. Organ Weights - No effects of MTA treatment were evident on the weights of the adrenals, kidneys, liver, lung, ovary, or testes.

7. Necropsy and Histopathology - [The histopathology report was prepared by Dr. Ward R. Richter of the Chevron Chemical Company. The samples were prepared by IRDC and shipped to Dr. Richter for evaluation.]

   The necropsy and histopathology reports discuss the local dermal reactions and the condition of the intestine regarding "mucoid enteritis" and the presumably associated "goblet cell hyperplasia of the colon."

   Dr. Richter concluded that there were no compound-related histologic changes. In particular, no changes in the structure of the optic nerve or liver related to MTA treatment were evident.

Conclusion:

This study is INVALID. The high rate of deaths on the test animals relative to the control group confound the interpretation of this study. It cannot be determined if MTA treatment caused the higher rate of deaths in the treated rabbits. Since so many of the rabbits had "mucoid enteritis," it is implied that unhealthy rabbits were used at the start of the study or some conditions resulted in the development of this condition during the study. It can be made apparent that once the "mucoid enteritis" condition develops, MTA seems to promote the death of the infected rabbits.

TB recognizes, however, that when survivors alone are considered, there was no development of lesions in the optic nerve or optic chiasma at dose levels up to and including 60 mg/kg/day.

Note: This study is also of limited usefulness because of the selection of 60 mg/kg/day as the high dose level. Since the purpose of the study was to assess for potential effects on the optic tract, a dose level known to affect the optic tract should have been selected.

Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: K. Dearfield
Mission Support Staff, Toxicology Branch (H7509C) 6.1.89

DATA EVALUATION REPORT

Study Type: 84-2 - Gene Mutation - Salmonella Asay

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-41

Test Material: Methylthioacetate (SX-1732)

Synonyms: MTA (CAS No. 1534-08-3)

Study Number(s): T5771.501014 (Microbiological Associates) and S-3108 (Chevron Chemical Company)

Sponsor: Chevron Chemical Company

Testing Facility: Microbiological Associates, Rockville, MD

Title of Report: Salmonella/Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with Methylthioacetate (SX-1732).

Author(s): T.E. Lawlor and V.O. Wagner, III

Report Issued: December 30, 1987

Conclusions:

MTA was not shown to be mutagenic at dose levels of up to and including 10,000 ug/plate in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence or absence of metabolic activation (S-9).

Classification: ACCEPTABLE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by James K. Burke attesting that four inspections consisting of protocol review, counting the plates, draft report, and draft to the final report were made.

REVIEW

In this study methylthioacetate (MTA) was tested for mutagenic activity against tester strains of Salmonella typhimurium histidine auxotrophy TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation by an S-9 mix prepared from rat liver microsomes. The experimental design consisted of testing five dose levels of MTA (100, 500, 2500, 5000, and 10,000 ug per plate) by exposing for a 48-hour incubation period at 37 degrees C after which the number of revertant colonies per plate were counted. An initial study was first performed and a confirmation study was also conducted. All dose levels of MTA, vehicle controls (DMSO), and positive controls were plated in triplicate. The following is a brief description of each of the tester strains used:

TA98: Reverts by mutagenesis at the his D3052 locus from histidine dependence (auxotrophy) to histidine independence (prototropy) by frame shift mutagens. This strain contains the pKM101 plasmid which further increases sensitivity, presumably by modifying a DNA polymerase complex. 2-Amino-anthracene and 2-nitrofluorene are used as the positive controls for this strain in the presence and absence of metabolic activation, respectively.

TA100: Is reverted by mutagens at his G46 locus which cause both frame shift and base substitution mutations. Like TA98, TA100 contains the pKM101 plasmid. 2-Aminoanthracene and sodium azide are the positive controls for this strain assay in the presence or absence of metabolic activation, respectively.

TA1535: Is mutated at the his G46 locus by mutagens that cause base substitutions. 2-Aminoanthracene and sodium azide are the positive controls for this strain in the presence or absence of metabolic activation, respectively.

TA1537: Is mutated at the his C3076 locus from histidine dependence by frame shift mutations. 2-Amino-anthracene and 9-aminoacridine are the positive controls for this strain in the presence or absence of metabolic activation, respectively.

TA1538: Is mutated at the his D3052 locus by frame shift mutagens. 2-Aminoanthracene and 2-nitrofluorene are the positive controls for this strain in the

presence or absence of metabolic activation, respectively.

[Note: All five strains in addition to susceptibility at either the his G46, his C3073, or his P3052 operons have additional mutation factors. One is the raf wall mutation which causes a loss of one of the enzymes responsible for the synthesis of part of the cell wall. The other is a deletion of the uvrB gene which results in a deficient DNA-excision-repair system.]

Overall, these strains are recognized to be sensitive, rapid, and accurate indicators of the mutagenic activity of a wide range of chemical classes. All strains were reported as being received directly from Dr. Bruce Ames of the University of California, Berkeley. Prior to use, the tester strain cultures were checked for their respective genetic markers. For example, the presence of the raf wall mutation was confirmed by demonstration of sensitivity to crystal violet. The presence of pKM101 plasmid was confirmed for tester strains TA98 and TA100 by demonstration of resistance to Ampicillin. In addition, the number of spontaneous reversion frequencies for each tester strain was determined and shown to be within the characteristic responses.

The test dose levels were based on information provided by the study sponsor (Chevron) which indicated that MTA was not toxic at dose levels up to 10,000 ug/plate. The plating procedures used were based on the procedures described by Maron and Ames (Mutation Research 113:173-215 (1975)).

Results:

There were no indications that MTA at dose levels up to and including 10,000 ug/plate resulted in a mutagenic effect in any of the five tester strains in either the presence or absence of S-9 metabolic activation.

Tables 21 and 22, photocopied from the study report, are attached to illustrate this result.

Conclusion:

This study is ACCEPTABLE. Sufficient data were generated to demonstrate that MTA is not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence or absence of S-9 metabolic activation.

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Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: K. Dearfield, Ph.D.
Mission Support Staff, Toxicology Branch (H7509C) 6.1.89

DATA EVALUATION REPORT

Study Type: 84-2 - Chromosome Aberration

TOX Chem No.: 584D 2A

Accession Number:

MRID No.: 405048-42

Test Material: Methylthioacetate (SX-1732)

Synonyms: MTA (CAS No. 1534-08-3)

Study Number(s): Chevron No. S-2891, Hazleton No. 2107-147

Sponsor: Chevron Chemical Company

Testing Facility: Hazleton Laboratories, Inc.

Title of Report: Clastogenic Evaluation of Methylthioacetate (SK-1732) in the Rat Bone Marrow Cytogenetic Assay Following a Four-Day Inhalation Pilot Study.

Author(s): James B. Terill, Ph.D., D.A.B.T.

Report Issued: November 12, 1987

Conclusions:

No evidence of chromosome aberrations at dose levels of 400 and 600 ppm of MTA (1.47 and 2.21 mg/L), dose levels which cause 20 percent decrease in body weights. Death results at 800 ppm.

Classification: UNACCEPTABLE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by K. Reilly attesting that four reviews (protocol review, study inspection, and final report (2) were made.

REVIEW

The basic study design consisted of exposing four groups of five male and five female rats (Fischer 344, obtained from the Charles River Laboratories, Raleigh, NC, about 9 weeks of age at start of exposure) to atmospheres containing either 0, 400, 600, or 800 ppm of methylthioacetate (MTA).

The test atmospheres were generated by putting undiluted test material (MTA) in gas wash bottles and passing air through the bottle and then into the test chamber. Prior to entering the chamber, the saturated atmosphere was diluted with air to produce the desired chamber atmospheric concentrations of MTA.

The rats were exposed to air containing MTA for 6 hours per day for four consecutive days and on the day following the fourth exposure (19 hours after exposure), the surviving rats were sacrificed and the bone marrow sampled for subsequent cytogenetic evaluation.

The bone marrow was assessed for possible clastogenic effects of MTA by first (2 1/2 hours prior to sacrifice) injecting the rat (IP) with 2.0 mg/kg of colchicine to arrest mitotic activity. The bone marrow was collected, treated with 0.075 M KCl, and prepared in fixative (methanol:acetic acid 3:1), dropped onto glass slides and stained with 5 percent Giemsa and dried. The slides were evaluated for at least twenty types of chromosome aberrations as indicated in the following list:

Chromatid gap	Pulverized chromosome
Chromatid break	Pulverized chromosomes
Isochromatid gap	Pulverized cells
Chromosome break	Ring chromosome
Chromatid deletion	Dicentric chromosome
Fragment	Minute chromosome
Acentric fragment	Double minute chromosome
Translocation	Abnormal metacentric chromosome
Triradial	Greater than ten aberrations
Quadriradial	Complex rearrangement

The report states that "50 spreads were read for each animal" and that a mitotic index based on at least 500 cells was calculated by scoring the number of cells in mitosis per 500 cells.

Results:

1. Chamber Atmosphere - The actual (analytical) exposure levels (assessed by a Miran 801 infrared analyzer) were shown to be within a few percent of the target

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concentrations. These atmospheric levels of 400, 600, and 800 ppm correspond to 1.47, 2.21, and 2.94 mg/L.

2. Mortality and Reactions - Only two female rats of all the rats in the high dose group (2.94 mg/L) survived the four exposures. Some of the signs of toxicity included red discharge from the nose and mouth, wheezing, polypnea and prostration, and urine-stained fur.

3. Body Weight and Food Consumption - Both mean body weight and food consumption were decreased for all groups of rats exposed to MTA. For example, mean body weights for both males and females exposed to 400 or 600 ppm were about 20 percent decreased. Total food consumption for the groups exposed to 400 ppm was 69 percent decreased for males and 61 percent decreased for females.

4. Clastogenic Evaluation - Because of the high rate of deaths in the group exposed to 800 ppm, only the rats from the group exposed to 0, 400, and 600 ppm were assessed for clastogenic effects of MTA.

   The study concluded that there was no significant effect of MTA on the percentage of cells with chromosomal aberrations in either of the two groups exposed to MTA. A copy of the summary table is attached to illustrate this finding.

Conclusion:

The classification is UNACCEPTABLE for this study of MTA effects on chromosome aberrations in vivo in the mouse bone marrow assay. There was no apparent effect on the mitotic index to indicate an effect of MTA on cell division. Since there was no effect, the 19-hour sampling time may be too long after the final exposure to assay all possible induced aberrations. This amount of time (19 hours) may be enough to allow cell division to occur which could lead to loss of some aberrations. Therefore, there may be an underestimate of the potential aberration frequency. It should be noted that there were slight increases in aberrations at the 600 ppm dose level, but these were apparently not statistically significant. Sampling time at 6 to 12 hours may have been more appropriate. It would also have provided useful additional information if the surviving animals in the high dose test group were analyzed for aberrations.

Toxicology Branch notes that a positive control should have been included in the study.

Although there were no apparent statistically significant increases in aberrations under these test conditions, this assay is considered UNACCEPTABLE for the reasons stated above.

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Reviewed By: J.D. Doherty 6/1/89
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: K. Dearfield, Ph.D.
Mission Support Staff, Toxicology Branch (H7509C) 6.1.89

DATA EVALUATION REPORT

Study Type: 84-4 - Mouse Micronucleus TOX Chem No.: 584D 2A

Accession Number: MRID No.: 405048-43

Test Material: Methylthioacetate (SX-1763, 99.21% purity)

Synonyms: MTA, CAS No. 1534-08-3

Study Number(s): S-2996

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center

Title of Report: Micronucleus Assay in the Mouse Bone Marrow Erythrocytes Following Inhalation Exposure to Methylthioacetate (SX-1763, 99.2% Purity).

Author(s): J.H. Carver, Ph.D. and L.C. Griffis, Ph.D., D.A.B.T.

Report Issued: January 15, 1988

Conclusions:

No micronuclei induced in male and female mice exposed to dose levels up to and including 796 ppm (3.00 mg/L) following 4 hours of inhalation exposure.

Classification: ACCEPTABLE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by B.M. Dowling, Quality Assurance Representative, attesting that reviews were made on two occasions.

REVIEW

In this study mice (male and female Swiss Albino, supplied by Simonsen Laboratories, Gilroy, California) were exposed to atmospheres containing 0, 445, 651, or 796 ppm of methylthioacetate (MTA, 99.2% purity from lot SX-1763). The mice were 49 to 50 days old at the start of exposure and they were exposed for 4 hours.

The test dose levels were selected based on a preliminary range-finding study in which mice were exposed to test atmospheres of 0, 788, 955, and 1060 ppm of MTA., Based on this study the LC_{50} was estimated to be 1005 ppm. Four of the five females and two of the five males died at the dose level of 1060 ppm and two of five males and females died at the dose level of 955 ppm.

The test material was generated into the chambers by bubbling air through glass gas washing bottles so that the air exiting from the second bottle was saturated with MTA. Variations of the chamber atmosphere were apparently made by adjusting the air flow. The atmospheric concentrations of MTA were monitored by means of a Miran-IA infrared gas analyzer which provided for testing the atmosphere by means of a probe located just above the mouse cages within the test chamber. The chamber atmospheres of MTA were plotted graphically versus time and the average concentration for the 240 minutes exposure period determined. The chamber atmospheres were reported as being very constant throughout the exposure period except for the high-dose group which had a period of about 24 minutes in which there was a marked dip (to about 400 ppm).

There were 15 mice of each sex in the control group (exposed to air). There were 18, 21, and 24 mice of each sex exposed to the low-, mid-, and high-dose test groups, respectively. More mice were included in the higher dose levels to compensate for any mice which might die as a result of exposure. The positive control group consisted of five mice of each sex that were dosed with triethylene melamine (TEM) intraperitoneally.

Five mice of each sex were selected for sacrifice at 24, 48, and 72 hours after the start of exposure. The positive control mice were sacrificed 24 hours after being dosed with TEM. Following sacrifice by cervical dislocation, the bone marrow was aspirated and prepared for microscopic analysis by smearing and fixing in methanol and stained with 2 percent Geisma for 45 minutes. Two bone marrow smears were made from each mouse.

One slide per mouse was selected for scoring. The second slide served as a backup. Initially 1000 polychromatic

erythrocytes (PCE) were evaluated at one place in the slide and then later a second 1000 PCE were evaluated. The ratio of normchromatic to polychromatic erythrocytes (NCE/PCE) and the number of micronucleated cells in both classes of erythrocytes were tallied. Criteria for a valid test included that spontaneous frequency of microchromatic (MN)-PCE must be $\leq$ 6 per thousand PCE and there must be evidence of mitotically active bone marrow. The data were analyzed for statistical significance using a one-tailed Fisher Exact Test.

Results:

1. The mice exposed to MTA in the main study showed signs of toxicity responses at all dose levels with signs of squinted or closed eyes, ocular discharge, and labored breathing being evident in the lowest test dose group. Higher levels resulted in ataxia and in the highest level deaths resulted (three females and males). The pathologist's report indicated that the lungs of all exposed mice contained lesions. The lesions consisted of hypertrophy and/or hyperplasia of the bronchiole epithelium in the mice with "minimal response to treatment." Chronic peribronchiolitis was a more common response and in some cases a more advanced form of suppurative pneumonia. The degree of severity of chronic peribronchiolitis did not display a dose response.

2. No cellular cytotoxicity was noted as the ratio of PCE: MNC did not appear to be altered.

3. The results of assessment for evidence of genetic toxicity are shown in the attached Tables 1 and 2 copied from the study report.

   These tables show no evidence of genetic toxicity of MTA based on a consistent increase in micronucleated polychromatic erythrocytes in bone marrow. Although there were a couple of statistically significant increases in the male mice, upon a second scoring, these increases do not appear biologically significant. The increases are within the background variability seen in Tables 1 and 2 (the experiment in Table 2 was to ascertain the background range of spontaneous micronuclei).

   The positive control (TEM) produced the expected positive response.

Conclusion:

This study is ACCEPTABLE. No evidence of micronuclei induced by MTA was generated by this study with test doses up to 796 ppm via inhalation. [Note: 445, 651, and 796 ppm correspond to 1.66, 2.44, and 3.00 mg/L, respectively.]

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