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

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: September 12, 2000

MEMORANDUM

SUBJECT: ***MSMA and DSMA:*** Data Evaluation Records and revised executive summaries

FROM: Anna B. Lowit, Toxicologist Anna B Lowit 9/12/00
Reregistration Branch 2
Health Effects Division (7509C)

THROUGH: Pauline Wagner, Branch Chief Pauline Wagner 9/12/00
Reregistration Branch 2
Health Effects Division (7509C)

TO: Tom Myers
Special Review and Reregistration Division (7508C)

DP Barcode: D268900
Submission: S579557
Chemical: MSMA and DSMA
PC Code: 013803 and 013802

Toxicology studies performed with methanearsonic acid have been reviewed by A. Lowit and N. McCarroll. The Data Evaluation Records (DERs) and revised executive summaries are attached to this memo. EPA has previously accepted toxicity studies performed with methanearsonic acid for MSMA and DSMA registration. Please store these DERs and revised executive summaries under PC codes for both chemicals (013803 and 013802).

DERs have been prepared for the following :

MRID #	Study Title
40632601	Fermenta Plant Protection Co. (1988) Justification for Dose Selection in New Methanearsonic Acid (MAA) Mouse Oncogenicity Study. Unpublished compilation. 184 p.
41651902	Chun, J.; Killeen, J. (1989) Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Metabolic Activation with Methanearsonic Acid (MAA): Lab Project Number: 89-0087: T8471.501014: 88-0223. Unpublished study prepared by Microbiological Associates Inc. and Ricerca, Inc. 169 p.
41651903	Chun, J.; Killeen, J. (1989) In Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells with Methaearsonic Acid (MAA): Lab Project Number: 89-0087: T8471.337001: 88-0220. Unpublished study prepared by Microbiological Assoceates Inc. and Ricerca, Inc. 127 p.
41651904	Chun, J.; Killeen, J. (1989) Mutagenesis Assay with Methanearsonic Acid: L5178Y TK+/-Mouse Lymphoma: Lab Project Number: 89-0087:T8471.701020: 88-0222. Unpublished study prepared by Microbiological Associates, Inc. and Ricerca, Inc. 159 p.
41651905	Chun, J.; Killeen, J. (1989) Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes with Methanearsonic Acid (MAA): Lab Project Number: 89-0087: T8471.380009: 88-0221. Unpublished study prepared by Microbiological Associates, Inc. and Ricerca, Inc. 130 p.
43178301	Schroeder, R. (1994) A Two-Generation Reproduction Study in Rats with Methanearsonic Acid (MAA): Final Report: Lab Project Number: 91/3668. Unpublished study prepared by Pharmaco LSR, Inc. 1954 p.

Revised executive summaries have been prepared for the following :

MRID #	Study Title
00159390	Rubin, Y. (1986) Methanearsonic Acid: Teratology Study in the Rabbit: PAL/006/MSM. Unpublished study prepared by Life Research Israel Ltd. 170 p.
40546101	Waner, T.; Nyska, A. (1988) Methanearsonic Acid: Fifty-two Week Chronic Oral Toxicity Study in Beagle Dogs: Document Number PAL/MAA/022. Unpublished study prepared by Life Science Research Israel, Ltd. 449 p.
41669001	Crown, S.; Nyska, A.; Waner, T. (1990) Methanearsonic Acid: Combined Chronic Feeding amd Oncogenicity Study in the Rat: Final Report: Lab Project Number: PAL/004/MAA. Unpublished study prepared by Life Science Research Israel Ltd. 1878 p.
41872701	Margitich, D.; Ackerman, L. (1991) Methanearsonic Acid: 21 Day Dermal Toxicity Study in Rabbits: Lab Project No: PH Unpublished study prepared by Pharmakon Research International Inc. 549 p.

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EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: DEVELOPMENTAL TOXICITY – RABBIT
[OPPTS 870.3700 (§83-3b)]
MRID NO. 15939001

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross for D.L. Fefee
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: H.T. Borges
Date: MAY 22 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

41926401 Mizens, M.; Killeen, J. (1990) A Teratology Study in Rats with Methanearsonic Acid: Lab Project Number: 89-3456: 89-0130. Unpublished study prepared by Bio/dynamics Inc., in cooperation with Ricerca, Inc. 490 p.

42010501 Wells-Gibson, N.; Marsh, D.; Krautter, G. (1991) Absorption, Distribution and Elimination of [Carbon 14]-Methyl MSMA in the Rat: Lab Project Number: 1344: 462E. Unpublished study prepared by East, Inc. 355 p.

42173201 Gur, E.; Pirak, M.; Waner, T. (1991) Methanearsonic Acid: Oncogenicity Study in the Mouse: Lab Project Number: PAL/023/MAA. Unpublished study prepared by Life Science Research Israel Ltd. 1680 p.

Supplement to Tox Document 011135, review for Accession 15939001, developmental toxicity study - rabbit. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 9/6/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 9/12/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit [870.3700 (§83-3b)]

DP BARCODE: D265953 SUBMISSION CODE: S579557
PC CODE: 013803 TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (>99.8% a.i.)

SYNONYM: Monosodium acid methanearsonate, MSMA

CITATION: Rubin, Y. (1986) Methanearsonic acid - teratology study in the rabbit. Life Science Research Israel, Ltd., Ness Ziona, Israel. Laboratory Report Number PAL/006/MSM, March, 30, 1986. MRID 15939001. Unpublished.

SPONSOR: Pamol Arad, Ltd., Tel-Aviv, Israel.

EXECUTIVE SUMMARY: In a developmental toxicity study, methanearsonic acid (purity >99.8%; Batch No. 107/84) was administered in distilled water by gavage to 14 mated New Zealand white rabbits per group at doses of 0, 1, 3, or 7 mg/kg/day on gestation days (GD) 7-19, inclusive. Subsequent groups of 13-14 mated New Zealand white rabbits were dosed with 0 and 12 mg/kg/day test material. On GD 29, surviving does were sacrificed and necropsied. Weights of uteri, and the number and locations of live and dead fetuses, early and late resorptions, implantations and corpora lutea were recorded. Fetal weights, crown-rump lengths, and external examination findings were recorded. All fetuses were subjected to fresh dissection, sexed internally, and processed and subjected to skeletal examination.

There were no treatment related deaths. Three animals (1 from control and 2 from 1 mg/kg/day group) died due to gavage error during the main study. Two females of the 12 mg/kg/day aborted and were killed on GD 25 and 29.

There was an increased incidence of orange discoloration of the urine in the 7 and 12 mg/kg/day groups (4 incidences in each group) compared to control (0 incidence). Increased incidence of soft feces and "few or no feces on undertray" at the 12 mg/kg/day dose level ($p<0.05$ or $p<0.01$) were also observed. A decrease in body weight gain (-76%) compared to control was observed during the dosing period for females in the 12 mg/kg/day group. Although maternal body weight change was decreased at the 7 mg/kg/day dose level for GD 7-8 and 10-13 intervals (29 and 63%

EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: DEVELOPMENTAL TOXICITY – RABBIT
[OPPTS 870.3700 (§83-3b)]
MRID NO. 15939001

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M. Signature:_____
Date: _____

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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PRIMARY REVIEWER: Steven Malish, Ph.D., Toxicologist
Section IV, Toxicology Branch II

SECONDARY REVIEWER: Susan Makris, M.S., Acting Section Head
Section IV, Toxicology Branch II

DATA EVALUATION REPORT

DEVELOPMENTAL TOXICITY STUDY

STUDY TYPE: Developmental Toxicity/Rabbits GUIDELINE: 83-3(b)

IDENTIFICATIONS: Submission: S444897 DP Barcode: D193345
MRID No.: 159390-01 Caswell No.: 295
P.C. Code: 013803 TRID No. 470199-004

TEST MATERIAL: Methanearsonic Acid

REGISTRANT: Pamol Arad, Ltd.
Tel-Aviv, Israel

TESTING LABORATORY: Life Science Research Israel, Ltd.
Ness Ziona, Israel

STUDY IDENTIFICATION: PAL/806/MSM

TITLE OF REPORT: Methanearsonic Acid
Teratology Study in the Rabbit

AUTHOR: Y. Rubin REPORT DATE: 3/30/86

SUMMARY: Inseminated New Zealand White rabbits [HY/CR] were administered Methanearsonic Acid [>99.8% a.i.] by oral gavage to 4 groups at doses of 0, 1, 3 or 7 mg/kg/day during days 7 thru 19 of gestation. Subsequently, a 5th group was dosed at 0 mg/kg/day and a 6th group dosed at 12 mg/kg/day for the same time period. Each group consisted of 13 to 14 animals.

less than controls), females in the 7 mg/kg/day actually gained 31% more weight during the dosing period than did controls.

Food consumption was decreased at 7 mg/kg/day for the GD 8-10 and 11-14 intervals (82 and 79% of controls, respectively; $p<0.01$) with a compensatory increase postdosing during the GD 20-23 interval (111% of controls; n.s.). At 12 mg/kg/day, food consumption was decreased for the GD 8-10, 11-14, 15-19, intervals (58-65% of controls; $p<0.001$), with compensatory increases postdosing during the GD 24-26 and 27-29 intervals (131-138% of controls; $p<0.01$). **The maternal toxicity LOAEL was 7 mg/kg/day, based on clinical signs (incidence of orange discoloration of the urine) and decreased food consumption. The maternal toxicity NOAEL was 3 mg/kg/day.**

There were no differences between the control and treated groups for number of corpora lutea, number of implantation sites, litter sizes, fetal sex ratios, fetal body weights, crown-rump lengths, or placental weights. There was a single incidence of total litter resorption at 7 mg/kg/day (all early resorptions), which resulted in increased mean early resorptions (1.0 vs. 0.4 for controls; n.s.); total resorptions were similar between groups. Although not observed at 3 mg/kg/day, increased pre-implantation loss was observed at 1 and 7 mg/kg/day (12.3, 19.0, 11.2, and 20.1% for 0, 1, 3, and 7 mg/kg/day groups, respectively; $p<0.001$) and at 12 mg/kg/day (15.1 vs. 10.5% for controls; $p<0.001$).

There were no treatment related effects on the occurrence of fetal external, visceral, or skeletal malformations. The total incidence of a 13th thoracic vertebra with ribs was increased in 12 mg/kg/day groups (1/97, 8/98, 3/124,1/95, 1/112, 13/80 fetuses from the control, 1, 3, 7, control-2, and 12 mg/kg/day groups); however the incidence at 3 and 7 mg/kg/day were similar to both set of controls. The total incidence of an 8th lumbar vertebra (5/97, 22/98, 7/124, 9/95, 27/112 and 51/80 fetuses from the control, 1, 3, 7, control-2, and 12 mg/kg/day groups, respectively; $p<0.001$).

The developmental toxicity LOAEL is 12 mg/kg/day, based on abortions and an increased incidence of skeletal variations (increased numbers of 13th thoracic vertebra with ribs and 8th lumbar vertebra). The developmental toxicity NOAEL is 7 mg/kg/day.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study in rabbits [OPPTS: 870.3700 (83-3b)].

1 hour and then injected i.v. with HCG to insure ovulation. The day of mating was designated as Day 0 of gestation.

D. Group Assignments and Dosage Level

Females were randomly assigned to the following groups prior to mating as per the following table.

Treatment Groups

Group	Treatment	Dosage[a] (mg/kg/day)	No. of Animals
1	Vehicle Control	0 (control)[b]	14
2	MAA	1	14
3	MAA	3	14
4	MAA	7	14
5	Vehicle Control	0	14
6	MAA	12	13

[a] Dosage Volume: 5 ml/kg/day
[b] Vehicle Control: Double distilled water
Groups 1-4 were run concurrently; Groups 5-6 were run concurrently

E. Preparation of Dose

The test material was formulated on each day of dosing as solutions in distilled water.

F. Analyses of the Test Substance for Active Ingredient

Analysis of the test material or dosing solutions were not included in the report.

G. Administration of Test Article

The test substance and the control were administered by oral gavage from days 7 to days 19 of gestation. The daily dose administered to each animal was based on the animal's body weight on the day of dosing. Doses were expressed in terms of the percentage active ingredient of the test material. The volume administered to all dose groups was 5 ml/kg.

H. Observations

All females were examined daily for signs ill-health or toxicity. Individual body weights were recorded on day 0 and 3, daily on days 7 thru 19, and on days 22, 25 and 29 of gestation. Individual food

Maternal toxicity at 12 mg/kg/day was characterized by abortion and decreases in mean absolute body weight and mean body weight gain and food consumption and at 7 mg/kg/day by decreases in mean body weight gain and food consumption. Developmental toxicity at 12 mg/kg/day was characterized by an increased incidence of skeletal variations, i.e., increased numbers of 13 thoracic vertebrae and ribs and 8th lumbar vertebrae.

Based on these results, the LOEL for maternal toxicity is 7 mg/kg/day; the NOEL for maternal toxicity is 3 mg/kg/day. The LOEL for developmental toxicity is 12 mg/kg/day; the NOEL for developmental toxicity is 7 mg/kg/day.

This study is classified as **Core Supplementary**. The study may be upgraded to minimum pending receipt and review of GLP statement, analytical data, if available, and maternal necropsy data.

I. OBJECTIVE:

The objective of this study was to assess the effects of Methanearsonic Acid on the embryonic and fetal development following oral administration to rabbits during the major period of organogenesis.

II. MATERIALS AND METHODS:

A. Test Material

Identity:	Methanearsonic Acid (MAA)
Batch No.:	107/84
Purity:	>99.8% a.i.
Description:	White crystalline solid
Storage:	Cool, well ventilated area

B. Test Animals

Species/Sex:	Female rabbits
Strain:	New Zealand White [HY/CR]
Body Weight [Gestation Day 0]:	≈3.0 to 3.2 kg
Identification:	Ear tags
Acclimation:	≈6 days
Housing:	Individually in metal cage
Food:	Altromin 2113 [pelleted high fiber rabbit breeding diet] ad libitum
Water:	Tap water ad libitum
Environment:	Temperature - 17 to 22°C.; Humidity - <45 to >75%; Air Changes - 15/hr; Light/Dark - 14 hr. light/10 hour dark
Source:	Charles River Laboratories, Italia

C. Mating

The females were mated naturally with males of the same strain and source when observed to be in spontaneous estrus. Each female was allowed to mate up to 3 times within a time span of approximately

fetuses (per fetus basis), number of affected litters	Fisher Exact test
(i) Individual litter proportions	Mann-Whitney U-test

Numbers of litters with one or more affected fetus were tested using the Chi square test (where all cells >5) or the Fishers exact test (where not all cells >5).

Group means and standard deviations were calculated for maternal body weight and food consumption for each day of measurement. Individual body weight and food consumption of animals that were not pregnant, died, aborted or contained no live fetuses at Day 29 were excluded from the group means. Net terminal body weight was calculated for each pregnant female by subtracting the weight of the gravid uterus from the body weight on Day 29.

The statistical methods do not clearly indicate how the duel control was utilized in the analysis of treatment group data.

M. Regulatory Compliance

A statement of quality assurance was signed and dated. No statements of No Confidentiality Claims, Good Laboratory Practice or Flagging were included in the report. Since the report includes a description of a deviation from GLP, it is assumed that the study was conducted under the principles of Good Laboratory Practice.

III. RESULTS:

A. Maternal Toxicity

a. Mortality

Three (3) animals died during the study, 2 from the 1 mg/kg/day [Group 2] and 1 from the Control [0 mg/kg/day] Group 5. Necropsy revealed the cause of death was due to dosing accidents.

b. Abortions

One (1) doe at 12 mg/kg/day [Group 6] aborted on day 25 of gestation and was sacrificed after aborting 1 dead fetus. The uterus contained 2 empty implantation sites, 2 early resorptions and 6 dead fetuses in varying states of autolysis. One other doe at 12 mg/kg/day [Group 6] aborted 8 dead and/or resorbing fetuses on day 29 and were sacrificed. The uterus contained 8 empty implantation sites.

consumption was measured twice weekly during the study for a 3 to 4 day period.

I. Termination

Females found dead, killed in extremis or showing signs of abortion during the study were necropsied. All surviving does were sacrificed [i.v. phenobarbital] on gestation day 29.

J. Cesarean Section

The contents of abdominal, thoracic and pelvic cavities were grossly examined. The reproductive tract was examined for gross lesions. The uterus was removed from the body, weighed and opened for internal examination, i.e., number of corpora lutea in each ovary, distribution of live and dead fetuses, and distribution of resorption sites [early or late] in each uterine horn. Pre- and post-implantation losses were calculated.

K. Fetal Examination

Each fetus and placenta was weighed; crown-rump lengths were measured. Each fetus was examined for external abnormalities, killed [s.c. phenobarbital], and carefully dissected. Visceral anomalies of individual fetuses were noted, and the fetuses were sexed. The skull of each fetus was sectioned transversely through the frontal-parietal suture and the brain examined. Each fetus was individually identified and fixed in ethanol for skeletal staining [Alizarin red S] and evaluation.

L. Statistical Evaluation

Presumed differences between control and treated groups were tested for statistical significance using the following tests.

a. Maternal Observations

Maternal Food Consumption, Body Weight and Body Weight Gain	Student "t" test
Maternal Clinical signs and Maternal Observations at Necropsy	Fishers Exact Test

b. Fetal Observations

Pre- and post observation loss	Mann-Whitney U-test
(i) Freeman-Tukey transformation	Student's "t" test
Fetal weight, length, placental weight	Student's "t" test
Skeletal evaluation, number of affected	Chi-square test or

Table 2. Maternal Mean Body Weight Change[a]

Gestation Day	Relative Maternal Body Weight[b] (gms)			
	Dose Level (mg/kg/day)			
	0	7	0	12
7 to 8	34	24 [-29[b]]	-3	-32 [*]
10 to 13	76	28 [-63]	70	46 [-34]
16 to 19	-28	68	45	31 [-31]
7 to 19[d]	180	235	246	59 [-76]
19 to 29	224	203	243	310 [28]
7 to 29	404	438	489	369 [-25]
0 to 29[e]	628	644	744	605 [-19]

[a]Adapted from original report, p. 24, 28, 29.
[b]Percentage change [] compared to the respective concurrent controls.
[*]Percentage not calculated due to sporadic decrease in the body weight of the treated group.
[d]Compound administered from days 7 thru 19 of gestation.
[e]Net terminal mean body weight change after subtracting weight of gravid uterus.

e. Food Consumption

Food consumption, showed decreases at days 8 to 19 of gestation [dosing period] at 3, 7 and 12 mg/kg/day with statistical significance being seen at 7 ($p<0.01$) and 12 ($p<0.001$) mg/kg/day for days 8 to 14 and for days 15 to 19 at 12 mg/kg/day vs. the controls (Table 3).

During the post-dosing period, food consumption at the 3 and 7 mg/kg/day levels was increased at days 20 to 23 and decreased, thereafter compared to the respective controls. Statistical significance was not seen. Food consumption at 12 mg/kg/day was decreased at days 20 to 23 and increased at days 24 to 29 ($p<0.01$) vs. the respective controls (Table 3).

Food consumption from days 0 to 29 at 3, 7 and 12 mg/kg/day showed slight decreases [not statistically significant] compared to the respective controls (Table 3).

c. Clinical Signs

Treatment-related clinical observations presented at 7 and 12 mg/kg/day consisted of soft feces, and an orange discoloration of the urine; "few or no feces on under-tray" were observed at 12 mg/kg/day. The incidence of animals with these findings was significant at the 12 mg/kg/day level (Table 1).

Table 1. Incidence of Gross Observational Signs During Gestation[a,b]

	Dose [mg/kg/day]					
Sign	0	1	3	7	0	12
Orange Discoloration of the Urine	0	0	0	4	0	4*
Soft Feces	2	2	0	2	0	7**
Few or no feces on undertray	0	2	0	0	0	5*

[a]Adapted from original report, p. 26.
[b]Non-pregnant animals excluded
*p<0.05
**p<0.01

d. Body Weight Changes

Absolute mean body weight showed a constant and statistically significant ($p<0.05$) decrease [≈5%] among 12 mg/kg/day animals from days 15 to 22 of gestation compared to the respective controls. Mean absolute body weight at study termination at this dose level and at all time intervals at the other dose levels were similar to the respective controls.

No changes were seen in the mean body weight gain that occurred at 1 and 3 mg/kg/day. At 7 mg/kg/day, decreases in the relative body weight of 29 and 63% occurred, respectively, at day 7 to 8 and 10 to 13 vs. the controls. Relative weights at the other time periods were comparable to the respective controls or difficult to interpret due to a weight loss in controls on days 16-19 (Table 2).

At 12 mg/kg/day, decreases in relative body weight were seen during the dosing period intervals [days 7 to 8, 10 to 13 and 16 to 19] and resulted in a decrease during the entire dosing period [days 7 to 19] of 76%. Relative body weight was increased after dosing at days 19 to 29 [28%] but the relative body weight was still decreased at days 7 to 29 and 0 to 29 vs. the respective controls (Table 2).

Table 4. Cesarean Section Observations[a]

Observations	Incidence of Observations — Dose Level [mg/kg/day]					
	0	1	3	7	0	12
# Assigned	14	14	14	14	14	13
Females Gravid (%)	12 85.7	14 100	14 100	14 100	14 100	13 100
Maternal Wastage						
# Died [%]	0	2	0	0	1	0
# Aborted [%]	0	0	0	0	0	2 [15.4]
# Non pregnant [%]	2	0	0	0	0	0
Corpora Lutea (Mean±SD)	10.6±2.0	10.9±3.0	10.6±2.4	10.2±1.7	10.8±2.4	9.9±1.4
Total Live Fetuses (#)	97	98	124	95	112	80
Live Fetuses (Mean±SD)	8.1±2.9	8.2±2.4	8.9±2.6	7.3±1.4	8.6±1.9	7.3±1.5
Total Resorptions (#)	17	7	8	18	15	15
Early Resorptions (Mean±SD)	0.4±0.5	0.3±0.5	0.1±0.3	1.0±1.5[b]	0.1±0.3	0.6±0.7
Late Resorptions (Mean±SD)	1.0±2.0	0.3±0.7	0.5±0.9	0.3±0.6	1.1±1.3	0.7±1.0
Pre-implantation Loss (%)	12.3	19.0***	11.2	20.1***	10.5	15.1***
Post-implantation Loss (%)	15.3	8.3**	6.8***	17.6	11.2	15.3
Sex Ratio [♂/♀]	49/48	56/42	55/69	53/42	66/46	41/39
(% Males)	50.5	57.1	44.4	55.8	58.9	51.3
Mean Fetal Weight (gm)	43.5	46.1	44.1	45.4	44.0	44.7
Mean Crown-Rump Length (mm)	92.9	95.9	93.7	94.2	94.7	94.4
Mean Placental Weight (gm)	5.5	6.0	5.5	5.8	5.5	5.6

[a] Adapted from original report, p. 30, 75 to 80.
[b] Total early resorptions occurred in 1 animal.
** $p \leq 0.01$, *** $p < 0.001$

B. Developmental Toxicity

Fetal malformations are summarized in Table 5 and fetal variations are summarized in Table 6.

a. External Examinations

External malformations were noted in two fetuses. In one control (Group 1) fetus, acrania, anencephaly, anophthalmia and cleft lip and face were seen (Table 5).

Table 3. Food Consumption During Gestation

Dose mg/kg/day	Food Consumption [gm/animal/day]								
	Gestation Day								
	1-4	5-7	8-10	11-14	15-19	20-23	24-26	27-29	0-29
0	217	246	249	237	230	198	185	168	215
1	220	242	242	232	230	224	179	159	216
3	207	242	234	220	215	208	164	154	207
7	200	234	205**	188**	218	219	183	165	205
0	241	255	251	243	259	233	177	167	229
12	244	258	162***	142***	167**	212	232**	231**	203

**p<0.01; ***p<0.001

f. Macroscopical Examination

Maternal macroscopic pathology data were not included in the report.

g. Reproduction Data

The pregnancy rate was 85.7% and 100% at the 0 and 1, 3 and 7 mg/kg/day groups, respectively, and 100% at 0 and 12 mg/kg/day. Day 29 Cesarean-sectioning observations were based on 12 pregnant dams at 0 mg/kg/day, 14 pregnant dams at 3, 7 and 0 [Group 5] mg/kg/day and 13 dams at 12 mg/kg/day and excluded the 2 dams at 0 mg/kg/day [Group 1] that were not pregnant, the 2 dams at 1 mg/kg/day [Group 2] and the 1 dam at 0 mg/kg/day [Group 5] that died from dosing accidents and the 2 dams at 12 mg/kg/day that aborted (Table 4).

There were no statistically significant differences among the dose groups in the litter means for: corpora lutea, litter sizes, sex ratio, fetal body weights, crown-rump length or placental weights. Early resorptions showed a non-statistical significant increase at 7 mg/kg/day [1.0] vs. the Group 1 control [0.4] but the total and late resorptions were similar [or less] than the controls (Table 4).

The increase in the mean number of resorptions at 7 mg/kg/day was attributed to a single incidence of total litter death [early resorptions]. The overall frequency of post-implantation loss was only slightly increased [17.6 vs. 15.3 in the Control] at this dose or at 12 mg/kg/day [15.3 vs. 11.2 in the Control] (Table 4).

Decreases in post-implantation loss occurred at 1 ($p<0.01$), and 3 ($p<0.001$) mg/kg/day and were judged not to be treatment related. Increases in pre-implantation loss occurred at 1 ($p<0.001$), 7 ($p<0.001$) and 12 ($p<0.001$) mg/kg/day but not at 3 mg/kg/day; no dose relationship was seen (Table 4).

Table 5. Summary of Fetal Malformations[a]

Observations	No. of Fetuses / No. of Litters					
	Dose Level (mg/kg/day)					
	0	1	3	7	9	12
No. Examined Externally	97/12	98/12	124/14	95/13	112/13	80/11
Acrania, anencephaly, anophthalmia, cleft lip, face	1/1[b]	0/0	0/0	0/0	0/0	0/0
Umbilical Hernia	0/0	0/0	0/0	1/1[c]	0/0	0/0
No. Examined Viscerally	97/12	98/12	124/14	95/13	112/13	80/11
Aortic arch markedly enlarged; pulmonary artery rudimentary	0/0	1/1	0/0	0/0	0/0	1/1
Lateral ventricles enlarged	0/0	3/2	0/0	0/0	0/0	0/0
Severe, internal, Hydrocephalus	0/0	0/0	0/0	1/1[c]	0/0	0/0
No. Examined Skeletally	97/12	98/12	124/14	95/13	112/13	80/11
Cranial malformation associated with acrania and anencephaly	1/16	0/0	0/0	0/0	0/0	0/0
Cranial malformation associated with severe internal hydrocephalus	0/0	0/0	0/0	1/1[c]	0/0	0/0

[a]Adapted from original report, p. 33, 36, 40, 44.
[b]Observed in same fetus.

Increased total incidences of the presence of an extra (8th) lumbar vertebrae [$p<0.001$] or (13th) thoracic vertebrae with ribs ($p<0.05$) were observed at 1 and 12 mg/kg/day vs. the respective controls. Only the mean percent of affected fetuses per litter for the 8th lumbar vertebrae showed statistical significance ($p<0.05$) vs. the control (Table 6). Statistical comparisons per litter incidence was not performed; however, the percent of litters affected with extra vertebrae at the 12 mg/kg/day level was increased. For the thoracic vertebrae 45.5% of the litters were affected [versus 8.3% or 7.7% min the control groups 1 and 5] and for the lumbar vertebrae 91% of the litters were affected [versus 33.3% or 61.6% in groups 1 and 5]. This suggests an affect of treatment at the highest dose level (Table 6).

One fetus in Group 4 (7 mg/kg/day) showed umbilical hernia. These malformations were not attributed to treatment with methanearsonic acid (Table 5).

No other external effects were seen.

b. Visceral Examinations

Visceral malformations included the following:

Hydrocephalus was noted in a single fetus [7 mg/kg/day] with umbilical hernia as noted above under external examination (Table 5).

Three fetuses of 2 Group 2 animals (1 mg/kg/day) litters were found to have enlarged lateral ventricles of the brain (Table 5).

A circulatory malformation occurred in two fetuses, one from each of Groups 2 (1 mg/kg/day) and 6 (12 mg/kg/day). In each case the aortic arch was prominently enlarged (2 to 3 times normal diameter) and the pulmonary artery was rudimentary. Neither the incidence or distribution of these various visceral malformations were indicative of a treatment-related effect (Table 5).

Observations of the viscera revealed variations, i.e. enlarged or hypotropic gallbladder, dilated ureters, hydronephrosis, ascites, hemorrhagic condition or discoloration of various organs at comparable [or lower] incidences in the treated vs. the controls animals.

c. Skeletal Examinations

Two fetuses, one each in Group 1 (control) and 7 mg/kg/day dose levels showed cranial malformations which were associated with the hydrocephalus respectively noted above under the external and/or visceral examinations (Table 5).

Maternal toxicity was further evidenced at 12 mg/kg/day by a decrease in the rate of mean body weight gain during the dosing period [day 7 to 19] together with a decrease in the maternal food consumption at 12 mg/kg/day during the dosing period. Maternal toxicity similarly was presented at 7 mg/kg/day as a decrease in the rate of mean body weight gain at day 7 to 13 of gestation [beginning of the dosing period] which was correlated with a decrease in the maternal food consumption throughout the dosing period.

No treatment related differences were seen in the cesarean section parameters. An increase in pre-implantation loss occurring at 1, 7 and 12 mg/kg/ day [but not at 3 mg/kg/day] was not considered to be test compound related for (i) true pre-implantation loss could not have been caused by exposure to the test material because dosing started after implantation had occurred and (ii) very early post-implantation loss is not always detectable at necropsy or may have been included in the pre-implantation loss.

A single incidence of total litter death (total early resorptions) occurred among a single female at 7 mg/kg/day. This phenomena was not considered to be treatment-related for the overall incidence of post-implantation loss did not show statistical significance at this dose or the next higher dose [12 mg/kg/day].

Fetal body weight was not affected by treatment.

The developmental parameters [external, visceral or skeletal] were similar in both the control and treated groups except for: (a) an occurrence of an increased number of 8th lumbar vertebrae and 13 pairs of thoracic ribs and vertebrae which was observed more frequently in the fetuses and litters at 1 and 12 mg/kg/day. In groups 1 thru 4 [0 thru 7 mg/kg/day], no dose relationship occurred for either lesion. The increased incidence of both variations at 1 mg/kg/day, therefore, was considered sporadic and of no toxicological significance. When the 12 mg/kg/day group was compared to the concurrent control, increases in both skeletal variations were seen in both the fetus and the litter incidences, with the increase in the fetal thoracic and lumbar vertebrae incidence showing statistical significance. This effect at 12 mg/kg/day was considered to be treatment related since (i) statistically significant alterations were seen in the fetus for both variations (ii) and the litter incidence was increased compared to the corresponding controls.

(b) Circulatory malformations observed at 1 and 12 mg/kg/day were not considered to be test compound related for (i) no dose-response was seen, (ii) only 1 fetus/litter was affected at each dose level and (iii) the malformation noted in the performing laboratories historical control showed 1 fetus/65 litters affected in 5 control groups, which was similar to the present study [i.e. 1 fetus/74 litters].

Table 6. Summary of Selected Fetal Variations*

Observations	No. of Fetuses/No. of Litters					
	Dose Level [mg/kg/day]					
	0	1	3	7	0	12
No. Examined Skeletally	97/12	98/12	124/14	95/13	112/13	80/11
13 thoracic vertebrae and 13 pairs of thoracic ribs	1/1	8*/2	3/3	1/1	1/1	13‾/5
8th lumbar vertebrae	5/4	22‾/8*	7/5	9/5	27/8	51‾/10

*Adapted from original report, p. 37, 41.
*p<0.05, ‾p<0.001 [chi-square test]; +p<0.05 [Mann-Whitney U-test performed on the mean percent of affected fetuses per litter].

Typical skeletal variations of the skull bones, ribs, hyoid, sternebrae, pelvis, and limbs were noted at non-significant levels among all control and treated groups without suggestion of a dose-response [not presented in the DER].

IV. DISCUSSION:

A GLP statement and maternal necropsy data was not included in the report.

Analytical data for either the technical or the dosing solutions were not presented. The reviewer is of the opinion that this omission would not compromise the study. Methanearsonic Acid is a relatively simple organic salt of arsenic and was highly purified according to the label. Moreover, a dose relationship was observed indicating that the concentration of the dosing solutions was, at least, qualitatively correct.

In this particular study, dosing was carried out from days 7 to 19 rather than the guideline [§ 83-3] recommended schedule of from days 6 to 18 [using day 0 as the day of mating]. The reviewer is of the opinion that this change would not significantly affect the outcome of the study.

Oral administration of Methanearsonic Acid at 0, 1, 3, 7 and 12 mg/kg/day to inseminated rabbits during days 7 to 19 of gestation resulted in significant maternal toxicity manifested by abortion on days 25 and 29 of gestation in 2 animals at 12 mg/kg/day. Even though the abortion incidence was not statistically significant compared to the controls, this effect was considered to be treatment related for (i) the abortions occurred in the high dose animals and (ii) a similar effect was reported to have occurred in the range-finding study [The reviewer notes that the range-finding study was not evaluated by the Agency].

Page_____ is not included in this copy.

Pages 22 through 37 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

(c) Enlarged lateral ventricles observed at 1 mg/k/day were not considered to be treatment related due to a lack of a dose response.

(d) Umbilical hernia, and hydrocephalus at 7 mg/kg/day [Group 4] was not considered to be treatment related since no dose relationship was seen.

V. CONCLUSIONS:

Methanearsonic Acid did not induce maternal or developmental toxicity at 1 and 3 mg/kg/day. At 7 mg/kg/day, maternal toxicity occurred while at 12 mg/kg/day maternal and developmental toxicity was presented.

Maternal toxicity at 12 mg/kg/day was characterized by abortion and decreases in mean absolute body weight and mean body weight gain and food consumption and at 7 mg/kg/day by decreases in mean body weight gain and food consumption. Developmental toxicity at 12 mg/kg/day was characterized by an increased incidence of skeletal variations, i.e., increased numbers of 13 thoracic vertebrae and ribs and 8th lumbar vertebrae.

Based on the results of this study the following NOELs and LOELs are established:

Maternal Toxicity	Developmental Toxicity
NOEL: 3 mg/kg/day	NOEL: 7 mg/kg/day
LOEL: 7 mg/kg/day	LOEL: 12 mg/kg/day

VI. CORE CLASSIFICATION: Supplementary; may be upgraded to minimum pending receipt and review of GLP statement, analytical data, if available, and necropsy data.

EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: CHRONIC ORAL TOXICITY FEEDING–DOG [OPPTS 870.4100 (§83-1b)] MRID NOS. 40546101/41266401

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M. Signature:_____
Date: _____

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

EXECUTIVE SUMMARY

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(3)

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: CHRONIC ORAL TOXICITY FEEDING–DOG
[OPPTS 870.4100 (§83-1b)]
MRID NOS. 40546101/41266401

Prepared for

Health Effects Division
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1921 Jefferson Davis Highway
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Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross for D. L. Fefee
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: H T Borges
Date: MAY 22 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above. 38

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

to control. Body weight gain in females was significantly decreased beginning at week 26 in the 8 mg/kg/day group (40-58% of controls; $p<0.05$) and throughout the study at 35 mg/kg/day (15-41% of controls; $p<0.05$). There was a marginal treatment related effect on food consumption (<5% less than controls; n.s.) in females at 8 and 35 mg/kg/day.

Changes in hematology and clinical chemistry parameters noted were sporadic, were not consistent over time and/or did not exhibit a dose-response pattern.

No toxicologically relevant differences in absolute organ weights occurred between treatment groups. In male dogs, the relative adrenal weight and relative liver weight were increased in the 35 mg/kg/day group (+29% and +9% for adrenal and liver, respectively, $p < 0.05$). The relative heart weight was increased in male and female dogs of the 35 mg/kg/day group (+17% and +27%, respectively, $p < 0.05$). Relative kidney weights were significantly increased in females of the 8 mg/kg/day group (+17%) in both sexes of the 35 mg/kg/day group (+15% M, +18% F). Moderate tubular nephrosis characterized by small vacuolation in the epithelial cells was noted in females of the 8 and 35 mg/kg/day groups (0/5, 1/5, and 2/5 for control, mid-, and high- dose groups, respectively). The only treatment related gross necropsy finding was a reduction in "abdominal fat pads" of a single high-dose male.

The incidence of estrous in females dogs was noted. The cumulative estrous incidence was 36, 50, 48, and 17 for control, 2, 8, and 35 mg/kg/day dose groups (+38%, +33%, and -47%, respectively). Based on the general poor health of females dogs in the 35 mg/kg/day dose group, this decreased incidence of estrous is considered a secondary toxic effect. Histopathology findings of the female reproductive system included an absence of corpora lutea in the 35 mg/kg/day group (3/5 vs. 0/5 control females).

The LOAEL was 8 mg/kg/day based on body weight gain and kidney effects (organ weight and histopathology) in females and clinical signs (severe diarrhea, vomiting, and excessive salivation) in both sexes. The NOAEL was 2 mg/kg/day.

This study is classified **Acceptable/Guideline** and satisfies the requirements for a chronic oral toxicity study in dogs [OPPTS 870.4100 (§83-1b)].

Supplement to Tox Document 011135, review for Accession 40546101/41266401, 52-week oral toxicity study-dog. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 8/28/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 8/30/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 52-Week Oral Toxicity - Dog [OPPTS 870.4100 (§83-1b)]

DP BARCODE: D265953
PC CODE: 013803

SUBMISSION CODE: S579557
TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (>99.8% a.i.)

CHEMICAL NAME: Monosodium acid methanearsonate (MSMA)

CITATION: Waner, T., A. Nyska (1988) Methanearsonic acid - fifty two week chronic oral toxicity study in beagle dogs. Life Science Research Israel, Ltd., P.O. Box 139, Ness Ziona 70 451 Israel. Laboratory Report Nos. LSRI Project Number PAL/MAA/022, January 25, 1988; LSRI Project Number PAL/008/MAA [Amendment], August 1, 1989. MRID 40546101 and 41266401. Unpublished.

SPONSOR: Pamol, Ltd. (for MAA Task Force), P.O. Box 13, Tel Aviv, Israel.

EXECUTIVE SUMMARY: In a chronic oral toxicity study (MRIDs 40546101 and 41266401), methanearsonic Acid (>99.8% a.i., Batch No. 107/84) was administered to 5 purebred beagle dogs (CPB-DCBE-67)/sex/group by capsule at dose levels of 0, 2.5, 8, or 40 mg/kg/day for one week and at dose levels of 0, 2, 8, and 35 mg/kg/day for 51 weeks. The following were examined in this chronic study: clinical signs, body weight, food consumption, ophthalmoscopy, neurology (including reflexes, postural reactions, clinical signs, behavior), clinical chemistry, hematology, urinalysis, gross pathology, organ weights, and histopathology.

There were no treatment related effects on mortality, ophthalmological, or neurological examinations. Beginning at week 1, treatment related clinical signs included vomiting, diarrhea, excessive salivation and sporadic anorexia were observed. Diarrhea and increased vomiting were observed in increased incidence in all dose groups in both males and females throughout the duration of the study. Compared to control dogs, excessive salivation was increased in dogs of the 8 and 35 mg/kg/day groups compared to controls.

In males of the 35 mg/kg/day group, body weight gain (-34-50%; $p<0.05$ or $p<0.01$) was decreased compared to controls starting at week 26. In females, body weights were decreased in the 8 and 35 mg/kg/day groups starting at week 26 (17-18% and 8-11% respectively) compared

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

JUL 27 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Methanearsonic Acid: Review of a 52 Week Toxicity Study/Dog and a Developmental Toxicity Study/Rabbit

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 7/22/94
Tox. Branch II, Review Section IV
HED (7509C)

TO: Virginia Dietrich, Product Manager (51)
Ron Kendall - PM Team Reviewer
Reregistration Division (7508W)

THRU: Susan L. Makris, M.S., Acting Section Head
Tox. Branch II, Review Section IV Susan L Makris 7/22/94
HED (7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 7/25/94
Tox Branch II; HED (7509C)

Task Identifications: Submission: S444897 DP Barcode: D193345
P.C. Code: 013803 Caswell No.: 295

ACTION REQUESTED: Review of [§ 83-1] 52 Week Chronic Oral Toxicity/Dogs [MRID NO.: 405461-01/412664-01] and [§ 83-3b] Developmental Toxicity/Rabbits [MRID 15930-01]

Data Evaluation Reports for the above referenced studies are attached. Summaries are provided below.

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Attachment 1

The following attachment is not available electronically
See the file copy.

Maternal toxicity at 12 mg/kg/day was characterized by abortion and decreases in mean absolute body weight and mean body weight gain and food consumption and at 7 mg/kg/day by decreases in mean body weight gain and food consumption. Developmental toxicity at 12 mg/kg/day was characterized by an increased incidence of skeletal variations, i.e., increased numbers of 13 thoracic vertebrae and ribs and 8th lumbar vertebrae.

Based on these results, the LOEL for maternal toxicity is 7 mg/kg/day; the NOEL for maternal toxicity is 3 mg/kg/day. The LOEL for developmental toxicity is 12 mg/kg/day; the NOEL for developmental toxicity is 7 mg/kg/day.

This study is classified as Core Supplementary. The study may be upgraded to minimum pending receipt and review of GLP statement, analytical data, if available, and maternal necropsy data.

1. [§ 83-1] 52 Week Chronic Toxicity in Dogs

Methanearsonic Acid (>99.8% a.i.) was administered by oral capsule to pure-bred Beagle dogs (5 dogs/sex) at 0 (Control), 2.5, 8 and 40 mg/kg/day for 1 week. From weeks 2 thru 52, dose groups were administered 0, 2, 8 and 35 mg/kg/day.

Decrease body weight gain occurred in the male at 35 mg/kg/day and in the female at 8 and 35 mg/kg/day.

A markedly increased incidence of diarrhea and vomiting occurred at the high dose level in both sexes while lower dose levels showed mild incidences compared to the respective controls. Excessive salivation occurred at the mid and high dose levels in both sexes. Anorexia was seen sporadically at the high dose level in both sexes.

At 35 mg/kg/day, the incidence of estrus was decreased compared to the Control.

Urine volume decreases with increases in the specific gravity occurred at 8 and 35 mg/kg/day; in females, only urine volume was decreased at 35 mg/kg/day.

Pathological [gross and histopathology] changes observed resulted from the marked weight loss and debility. The females at 35 mg/kg/day presented an absence of corpora lutea, a single animal presented an absence of hepatic glycogen and animals at both 8 and 35 mg/kg/day showed nephrosis. A single male at 35 mg/kg/day showed a reduction in the abdominal fat pads.

Based on these results, the LOEL for chronic toxicity was 8 mg/kg/day. The NOEL for chronic toxicity was 2 mg/kg/day.

The study is classified as Core Guideline and satisfies the data requirement [§ 83-1] for a chronic toxicity study in dogs.

2. [§ 83-3b] Developmental Toxicity Study in Rabbits

Inseminated New Zealand White rabbits [HY/CR] were administered Methanearsonic Acid [>99.8% a.i.] by oral gavage to 4 groups at doses of 0, 1, 3 or 7 mg/kg/day during days 7 thru 19 of gestation. Subsequently, a 5th group was dosed at 0 mg/kg/day and a 6th group dosed at 12 mg/kg/day for the same time period. Each group consisted of 13 to 14 animals.

At 35 mg/kg/day, the incidence of estrus was decreased compared to the Control.

Urine volume decreases with increases in the specific gravity occurred at 8 and 35 mg/kg/day; in females, only urine volume was decreased at 35 mg/kg/day.

Pathological [gross and histopathology] changes observed resulted from the marked weight loss and debility. The females at 35 mg/kg/day presented an absence of corpora lutea, a single animal presented an absence of hepatic glycogen and animals at both 8 and 35 mg/kg/day showed nephrosis. A single male at 35 mg/kg/day showed a reduction in the abdominal fat pads.

Based on these results, the LOEL for chronic toxicity was 8 mg/kg/day. The NOEL for chronic toxicity was 2 mg/kg/day.

The study is classified as Core Guideline and satisfies the data requirement [§ 83-1] for a chronic toxicity study in dogs.

I. INTRODUCTION:

The study was designed to test the possible chronic toxicity associated with dietary feeding of the test compound to dogs for 52 Weeks.

II. MATERIALS:

A. Test Material

Chemical:	Methanearsonic Acid
Synonym:	MAA
Grade:	Technical
Batch:	107/84
Purity:	>99.8% a.i.
Description:	White crystalline powder
Storage:	Sealed container, room temperature in dark
Stability:	Stable under laboratory storage conditions

B. Test Animals:

Species:	Dog
Strain:	Beagle (CPB-DCBE-67)
Age:	5 months old at initiation
Groups:	5 groups of 4 animals/sex
Weight:	Male: 7.4-13.6 Kg; Female: 6.7-11.7 Kg [at arrival]
Acclimatization:	≈1 month
Housing:	1 animal/sex/pen
Feed:	400 gm/animal/day of a complete pelleted dog diet [Dog Breeding/Maintenance Diet, #4134 [Altromin

C11135

Reviewed by: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 7/21/94
Review Section, IV; Toxicology Branch II (7509C)
Secondary Reviewer: Susan L. Makris, M.S., Acting Section Head
Review Section, IV; Toxicology Branch II (7509C) Susan L Makris 7/22/94

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity Study/Dog [§ 83-1]

MRID NO.: 405461-01/412664-01 **DP Barcode:** D193345

P.C. CODE: 013803 **Caswell No.:** 549A

TEST MATERIAL: METHANEARSONIC ACID

SYNONYMS: MAA

STUDY NO.: LSRI Project Number PAL/MAA/022
LSRI Project Number PAL/008/MAA [Amendment]

SPONSOR: Pamol, Ltd. (for MAA Task Force)
P.O. Box 13
Tel Aviv, Israel

TESTING FACILITY: Life Science Research Israel, Ltd.
P.O. Box 139,
Ness Ziona 70 451 Israel

TITLE of REPORT: Methanearsonic Acid
Fifty Two Week Chronic Oral Toxicity Study in Beagle Dogs

AUTHOR: T. Waner, A. Nyska

REPORT ISSUED: January 25, 1988; August 17, 1989 (Amendment)

EXECUTIVE SUMMARY:

Methanearsonic Acid (>99.8% a.i.) was administered by oral capsule to pure-bred Beagle dogs (5 dogs/sex) at 0 (Control), 2.5, 8 and 40 mg/kg/day for 1 week. From weeks 2 thru 52, dose groups were administered 0, 2, 8 and 35 mg/kg/day.

Decrease body weight gain occurred in the male at 35 mg/kg/day and in the female at 8 and 35 mg/kg/day.

A markedly increased incidence of diarrhea and vomiting occurred at the high dose level in both sexes while lower dose levels showed mild incidences compared to the respective controls. Excessive salivation occurred at the mid and high dose levels in both sexes. Anorexia was seen sporadically at the high dose level in both sexes.

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F. Statistical Analysis

The significance of any inter-group difference in growth performance, food consumption blood composition and organ weights was assessed by Student's t-test using a pooled within-group error variance. Organ weights were analyzed by the analysis of covariance using body weight at necropsy as the covariant.

G. Regulatory Compliance

A quality assurance statement and statements of compliance with Good Laboratory Practice Standards and of No Data Confidentiality Claims were signed and dated. No flagging statement was included in the report.

III. METHODS

A. Physical Examination

Each animal was subjected to a complete physical examination before dosing commenced and, thereafter, at approximately 2 week intervals. Particular attention was paid to the following:

Teeth and gums, mucous membranes and skin, ears (external auditory canal), superficial lymph nodes, abdomen - including palpation, external genitalia and mammary glands, chest including auscultation of heart and lungs, gait and stance including palpation of limbs, general behavior and appearance and body temperature.

B. Clinical Signs

Animals were examined for pharmacological and toxicological effects twice (2) daily on week days and daily on the weekends.

C. Body Weight

Each animal was weighed weekly throughout the acclimatization and study periods before the first daily meal.

D. Food Consumption

The weight of the food refused was measured daily and an estimate made of the amount of food spilled. Individual and mean weekly food intake were calculated. Anoretic animals fed extra food were deleted from the food consumption means during that week.

E. Ophthalmoscopy

Before commencement of the study and after 52 weeks of treatment, both eyes of all dogs were examined by means of an indirect binocular ophthalmoscope after the instillation of 0.5% Tropicamide[2].

Spezialfutterwerke, Lage, West Germany]

Water: Tap water ad libitum via automatic watering system

Environment: Air Temperature: 21±2°C.; humidity: not specified; Air changes: 15/hr; light cycle: 12 light/12 hours dark

Source: Central Institute for Breeding of Laboratory Animals TNO, Zeist, the Netherlands

C. STUDY DESIGN:

Animal Assignments

Twenty (20) dogs per sex were randomly assigned [by unique number] to 4 test groups and administered 0 (Control), 2.5, 10 and 40 mg/kg/day during week 1 by oral capsule. The dose levels were changed by the study sponsor after the animals had been on study for 1 week and from weeks 2 to 52 animals were administered 0, 2, 8 and 35 mg/kg/day of the test compound by oral capsule (Table 1).

Table 1. Animal Test Group Assignments

Group	Dose (mg/kg/day)*	Animals on Test	
		♂	♀
1	0	5	5
2	2	5	5
3	8	5	5
4	35	5	5

*Doses of 0, 2, 5, 10 and 40 mg/kg/day were used for the first week.

D. Test Material Dose Preparation and Administration

All dogs were dosed orally by capsule [size 000], once a day, 7 days a week. The test material was weighed directly into gelatin capsules. The weight of the compound required for daily administration to each dog was adjusted weekly within 24 hours of each animal weighing. Each control dog received an empty gelatin capsule.

E. Analysis of Test Material

A sample obtained from the bulk storage container was assayed to demonstrate stability after the completion of the study.

(1) Hematology

Hematology

Erythrocyte Count* [RBC]
Hematocrit* [PCV]
Hemoglobin* [Hb]
Leucocyte Count*
Leucocyte Differential Counts
Platelet Count*
Prothrombin Time
Mean Corpuscular Volume
Mean Corpuscular Hemog. Conc.
Erythrocyte Sedimentation Rate [ESR]

*Required by Guidelines

(2) Clinical Chemistry

Clinical Chemistry

Electrolytes	Other
Calcium* [Ca]	Albumin*
Phosphorous*	Globulin*
Chloride*	Albumin/Globulin Ratio
Sodium*	Total Protein*
Potassium*	Creatinine*
	Blood Urea Nitrogen*
Enzymes	Glucose*
	Total Bilirubin* [Bili]
Serum Alanine Aminotransferase* [ALT]	Cholesterol* [Chol]
Serum Aspartate Aminotransferase*	Triglycerides
Alkaline Phosphatase [ALP]	
Creatinine phosphokinase [CPK]	
Lactic Dehydrogenase [LDH]	

*Required by Guidelines

(3) Urinalysis

The following parameters were examined at approximately the same time as for clinical chemistry parameters [12, 25 and 51 weeks of treatment, ≈6 to 22 hours after dosing]. Food and water were withheld overnight prior to sample collections. Occasional contamination of the urine sample required resampling. The following parameters were measured.

F. Neurology

A full neurological examination was performed on all animals before commencement of treatment and at the last day of the dosing period. Reflexes tested and observations made included:

(1) Cranial Nerve Reflexes

Pupillary light and consensual light
Palpebral, blink and corneal
Gag
Startle (oculo-acoustic and auralacoustic)
General examination, e.g. for dropping facial muscles, atrophy of tongue, etc.

(2) Segmental Reflexes

Flexor (withdrawal) and crossed extensor
Patellar
Panniculus
Extensor tone

(3) Postural reactions

Placing reactions - visual and tactile
Extensor postural thrust
Righting reactions
Hopping Reflex

(4) General Observations

Behavioral changes, e.g. aggression, sedation
Abnormalities of gait and stance
Presence of tremor or other dyskinesia

G. Clinical Pathology

Blood samples were withdrawn from the jugular vein of each dog, 20-24 hours after dosing and after overnight fasting. Hematology and clinical chemistry examinations were carried out before commencement of treatment and after 12, 26 and 51 weeks of treatment. The following parameters were measured.

Organ Weights

ADRENALS	LIVER*
BRAIN* [+ BRAIN STEM]	OVARIES
HEART	TESTES*
KIDNEYS*	THYROID - PARATHYROIDS

*Required by Guidelines

(3) Histopathology

All tissues/organs listed below were evaluated for all animals.

Histopathology

Digestive System	Respiratory System
Salivary glands	Trachea
Esophagus	Lung
Stomach	
Duodenum	Cardiovascular/Hemo.System
Jejunum	
Cecum	Aorta
Colon	Heart[a]
Ileum	Lymph nodes[d]
Rectum	Spleen
Liver	Sternum with bone marrow
Pancreas	
Gall Bladder	Urinogenital System
Tongue	
	Kidneys[e]
Neurological System	Urinary bladder
	Testes with Epididymides
Brain[b]	Prostate
Pituitary	Uterus/Cervix
Peripheral nerve (sciatic)	Ovaries
Spinal cord[c]	
Eyes with optic nerve	Other
Glandular System	Skin
	Mammary glands (9)[f]
Adrenals	Lesions/masses
Parathyroids	Skeletal muscle
Thyroids	Tibia femoral joint
Thymus	

[b]Sectioned at - cerebellum, cerebral cortex, thalamic nuclei, mid-brain and medulla
[c]Prepared in transverse and longitudinal sections at cervical, thoracic and lumber levels
[a]Both auricular and ventricular areas
[d]Cervical, mesenteric and abdominal nodes
[e]Renal Sections were stained for lipids using Oil Red Oil stain
[f]Caudial and cranial areas

Urine Parameters

Appearance Volume* Specific Gravity* Sediment [Microscopic]* Protein* pH	Glucose* Turbidity Bilirubin Blood Pigments* Urobilinogen Ketones Total Red. Substances+

*Required by Guidelines
+Pre treatment and 12 weeks

G. Necropsy

After completing 52 weeks of treatment, and following an overnight fast, all animals were administered i.v. sodium pentobarbital anesthesia and sacrificed by rapid exsanguination. A necropsy for gross pathology was performed

(1) Gross Pathology

All necropsies included a detailed necropsy, involving opening of the cranial, thoracic and abdominal cavities. Any abnormalities seen were recorded.

The external features of the animal were scrutinised and compared to any relevant comments on the clinical history report. The first incisions allowed rapid preparation and fixation of costal bone marrow smear. The eyes, complete with optic nerve and relevant adnexa were removed. The cranial cap was lifted and the brain dissected free of meninges. The pituitary was freed from the sella turcica and fixed separately. The ventral abdominal and thoracic skin was reflected to allow observation of the subcutaneous structures, in particular. mammary glands and superficial lymph nodes. Abdominal and thoracic viscera were examined in situ and any abnormalities were noted. The serosal surface of the entire intestinal tract was examined after removal. The tract was then sectioned longitudinally and the mucosa examined. After weighing, the major organs were scrutinized and where appropriate, the cut surfaces were examined.

(2) Organ Weights

The following organs/tissues were weighed and organ weight/body weight ratio [relative organ weight] and organ weight/brain ratio were calculated for each animal at termination. In addition, organ weights were calculated by analysis of covariance using body weight as the covariant.

D. Clinical Observations

Clinical signs associated with dosing in both sexes were vomiting, diarrhea, excessive salivation and sporadic anorexia. In the high dose females, a decreased incidence of estrus was related to treatment.

Both sexes showed a dose related increase in the cumulative diarrhea incidence throughout the study at all time periods [except in the male at 2 mg/kg/day on weeks 1-4 and 13-16 vs. the Control]. By the end of the study, the cumulative incidence of diarrhea in the male was 4, 4 and 18 times that of the Control and in the female 2, 5 and 51 times that of the Control, respectively, at 2, 8 and 35 mg/kg/day. The incidence of diarrhea showed an increase in rate during weeks 23 to 28 in the males and weeks 33 to 48 in the females of the 8 and 35 mg/kg/day groups (Table 3).

The cumulative incidence of vomiting was increased in a time related manner in all treated dose levels in the male and in the female at 8 and 35 mg/kg/day compared to the Control. By the end of the study, the incidence of vomiting in the male was 6, 4, and 85 times that of the Control, respectively, and 2, 8 and 35 mg/kg/day and 4 and 15 times that of the Control at 8 and 35 mg/kg/day, respectively, in the female (Table 3).

The incidence of excessive salivation in males showed a time related increase at 8 and 35 mg/kg/day equivalent to 32 and 44 times that of the Control, respectively, at the end of the study. Females showed a time related increase in excessive salivation at 8 and 35 mg/kg/day equivalent to 150 and 600 times that of the control, respectively, by the end of the study (Table 3).

In females, estrus was observed at a 47% lower incidence at 35 mg/kg/day vs. the Control. However, the incidences at 2 and 8 mg/kg/day were higher than the controls (Table 3).

IV. RESULTS:

A. Analytical Chemistry

The two analyses performed after the completion of the study revealed that the percentage active ingredient was 99.1% and 99.75%, respectively. Stability of the test substance was, therefore, demonstrated for the duration of the study.

B. Mortality

No mortality occurred during the study.

C. Physical Examination

Poor physical condition [loss of weight] was the most prominent observation as evidenced by an increased incidence in the number of thin male animals at intervals throughout the study beginning with week 4. A single thin [cachectic] female was seen at 35 mg/kg/day at weeks 48 and 49. A dull and lusterless coat was more apparent in both sexes of the treated groups starting about week 20 of the study when compared to the Controls (Table 2).

Table 2. Physical Condition[a]

Physical Condition [Weeks]	Dose (mg/kg/day)							
	Males[b]				Females[b]			
	0	2	8	35	0	2	8	35
Thin								
14	0	1	0	0	0	0	0	0
26	0	1	0	3	0	0	0	0
40	0	2	1	4	0	1	0	2
52	0	2	0	4	0	0	0	1[c]
Dull/Lusterless Coat								
26	0	1	0	0	0	0	1	0
40	1	1	3	1	0	2	3	2
52	0	4	1	3	1	1	1	1

[a] Adapted from original report p. 77 to 104.
[b] 5 animals/sex at each dose level.
[c] A single animal was cachectic and anorectic during this approximate time period.

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Table 4. Mean Body Weight in Males[a]

Weeks on Test	Dose [mg/kg/day]			
	0	2	8	35
	Mean Body Weight [kg]			
1 [%]	10.1	10.2 [1]	10.3 [2]	10.1 [0]
13 [%]	12.0	11.7 [-3]	12.2 [2]	11.5 [-4]
26 [%]	12.2	11.7 [-4]	12.3 [1]	10.8 [-12]
39 [%]	13.4	12.8 [-5]	13.0 [-3]	11.6 [-13]
53 [%]	13.7	13.0 [-5]	13.4 [-2]	11.9 [-13]
Percentage Mean Body Weight Gain [%][b]				
1 to 13	19.2	14.9	18.3	13.9
1 to 26	21.2	15.4	20.0	7.3**
1 to 39	32.6	25.6	27.1	14.1*
1 to 53	36.3	27.9	30.9	18.3**

[a]Adapted from original report, p. 44, 105 to 109.
[b]Percentage change in body weight using mean of individual values [baseline = 1st week body weight]
*p<0.05; **p<0.01

Females showed decreases [>10%] in the mean body weight at 8 mg/kg/day starting at Week 26 and continuing to termination. Decreases of ≥10% occurred sporadically at 35 mg/kg/day at Weeks 26 and 53 compared to the respective controls. Statistically significant decreases in the mean body weight gain in females occurred at 8 mg/kg/day at Weeks 26 ($p<0.01$), 39 and 53 ($p<0.01$) and at 35 mg/kg/day at all time periods [$p<0.05$ to $p<0.001$] compared to the controls (Table 5).

Table 3. Cumulative Incidence of Clinical Signs[a]

Signs Observed (weeks)	Dose [mg/kg/day] 0	2	8	35
Diarrhea				
1- 4	1 [0[b]]	1[1]	6[4]	10[33]
13-16	7 [0]	8[6]	14[12]	32[95]
25-28	11 [3]	19[7]	29[22]	60[167]
49-52	14[10]	58[19]	51[50]	257[510]
Vomiting				
1- 4	0 [0]	10 [1]	1 [5]	20 [12]
13-16	0 [2]	12 [1]	6 [16]	78 [28]
25-28	3 [6]	15 [3]	11 [17]	144 [51]
49-52	3 [6]	17 [4]	13 [22]	254 [88]
Excessive Salivation				
1- 4	0 [0]	0 [0]	0 [4]	0 [11]
13-16	0 [0]	0 [0]	3 [39]	11 [55]
25-28	2 [0]	0 [0]	30 [107]	60 [309]
49-52	2 [0]	0 [0]	64 [150]	87 [600]
Estrus				
1- 4	[0]	[0]	[1]	[0]
13-16	[7]	[26]	[23]	[2]
25-28	[16]	[34]	[32]	[12]
49-52	[36]	[50]	[48]	[17]

[a]Adapted from the original report, p. 69 to 76 and Amendment p. 22 thru 26.
[b][]Females

E. Body Weight

Males showed a decrease [>10%] in the absolute mean body weight vs. the controls at 35 mg/kg/day starting a week 26 an continuing for the duration of the study. In males at 35 mg/kg/day, marked and statistically significant decreases in the mean body weight gain occurred at Weeks 26 and 39 ($p<0.05$) and 53 ($p<0.01$) compared to the controls. Mean body weight gain showed minimal decreases [not statistically significant or dose related] at 2 and 8 mg/kg/day compared to the respective controls [Table 4].

I. Clinical Pathology

(1) Hematology

Males showed marginal decreases in packed cell volume, hemoglobin and red blood cell parameters at 35 mg/kg/day at all time periods vs. the respective concurrent controls. Females showed marginal decreases of PCV, HgB and RBC at all time periods at 8 and 35 mg/kg/day, at Week 12 at 2 mg/kg/day for the above parameters and at Week 26 for RBC. One high dose male animal showed an elevated erythrocyte sedimentation rate during all examinations which resulted in higher values for this parameter at all time periods (Table 6).

Sporadic statistical significant changes were seen in the white blood cell parameters in both males and females throughout the study. These changes were considered to be of no toxicological signficance.

(2) Clinical Chemistry

Alkaline phosphatase values in males showed marginal increases at all dose levels at Weeks 12, 26, and 51 vs. the respective concurrent control. No dose-relation was seen. Sporadic statistically significant increases were seen at 2 mg/kg/day at Weeks 26 and 51. Females were considered to be not remarkable (Table 7, 8).

Lactic dehydrogenase in the male showed marginal increases at all dose levels and at all time intervals [except at 8 mg/kg/day at week 51] vs. the concurrent controls. No dose relationship was seen. In females, LDH was increased at 35 mg/kg/day on week 12 and at all dose levels at week 51 compared to the concurrent control; on week 26, a related decrease was seen vs.the control. No statistical significance was seen during the study (Table 7, 8).

Alanine aminotransferase showed a statistically significant ($p<0.05$) but marginal decreases at week 51 in males and females at 35 mg/kg/day vs. the concurrent controls (Table 7, 8).

Creatinine phosphokinase values in males showed marginal increases at all dose levels at Weeks 12 and 26 ($p<0.05$ at 35 mg/kg/day) and at Weeks 51 at 35 mg/kg/day vs. the concurrent controls. No dose relationship was seen during the study. Marginal increases in the CPK values for females were observed at the 35 mg/kg/day on weeks 12 and 51 (Table 7, 8).

Cholesterol values were decreased at 35 mg/kg/day for males and females at Weeks 13, 26 and 51; they were significantly lower at 35 mg/kg/day in the female [3.66 mmol/l, $p<0.05$] vs. the concurrent controls [4.65 mmol/l] at Week 51 (Table 7, 8).

Table 5. Mean Body Weight in Females[a]

Weeks on Test	Dose (mg/kg/day)			
	0	2	8	35
	Mean Body Weight (kg)			
1 (%)	10.4	9.7 (-7)	9.7 (-7)	11.2 (8)
13 (%)	12.0	11.3 (-6)	10.9 (-9)	11.9 (-1)
26 (%)	13.2	12.5 (-5)	10.9 (-17)	11.7 (-11)
39 (%)	14.2	13.6 (-4)	11.7 (-18)	13.0 (-8)
53 (%)	14.3	14.0 (-2)	11.9 (-17)	12.9 (-10)
Percentage Mean Body Weight (%)[b]				
1 to 13	16.4	15.5	11.3	6.4*
1 to 26	27.8	28.5	11.0**	4.3**
1 to 39	37.3	38.6	19.9*	16.5**
1 to 53	37.8	42.9	22.0*	15.5**

[a] Adapted from the original report, p. 44, 105 to 109.
[b] Percentage change in body weight using mean of individual values (baseline = 1st week body weight)
* $p<0.05$; ** $p<0.01$; *** $p<0.001$

F. Food Consumption

Dosing with MAA resulted in anorexia in 1 male and 2 females dogs at 35 mg/kg/day for variable periods of time. These animals were deleted from the weekly food consumption means. Food consumption was considered to be not remarkable at 2 and 8 mg/kg/day vs. the Control. Females at 8 and 35 mg/kg/day showed a marginal decrease (5%) in the mean weekly food consumption for weeks 1 to 52 vs. the Control; mean weekly food consumption values for the males were similar between treated and control groups.

G. Neurological Examination

Neurological examination was not remarkable.

H. Ophthalmoscopic Examination

Ophthalmoscopic examination was not remarkable.

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ other	__ other

S9 mix composition:

S9-fraction	0.10 mL
0.2 M $MgCl_2$/0.825 M KCl	0.04 mL
0.04 M NADP	0.10 mL
0.05 M Glucose-6-phosphate	0.10 mL
1.00 M NaH_2PO_4/K_2HPO_4 (pH 7.4)	0.10 mL
H_2O	0.56 mL

4. Test organisms: *S. typhimurium* strains

__ TA97 x TA98 x TA100 __ TA102 __ TA104
x TA1535 x TA1537 x TA1538;

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used:

Preliminary cytotoxicity test: (TA100 only, single plating)
Nonactivated and activated conditions:
10, 33, 67, 100, 333, 667, 1000, 3333, 6667, 10,000 µg/plate

Mutation assay: (all strains, triplicate plating)
Nonactivated and activated conditions:
667, 1000, 3333, 6667, 10,000 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:

x standard plate test
__ pre-incubation
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test

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TA100 in the preliminary cytotoxicity test. The solvent and positive controls produced the appropriate response in the respective tester strains. **There was no evidence that methanearsonic acid increased mutant colonies over background in eithr the presence or the absence of S9 activation.**

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5100[3] (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test aterial: Methanearsonic acid

   Description: white crystalline solid
   Lot/Batch #: 107/84
   Purity: 99.8% a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: deionized distilled water
   Other comments: none

2. Control materials

   Negative: none
   Solvent/final concentration: deionized distilled water
   Positive:
   Nonactivation:
   Sodium azide 1.0 µg/plate TA100, TA1535
   2-Nitrofluorene 1.0 µg/plate TA98, TA1538
   9-Aminoacridine ____ µg/plate
   ICR-191 2.0 µg/plate TA1537

   Activation:
   2-Aminoanthracene 0.5 µg/plate all strains

[3]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

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per plate over the corresponding solvent control value at any test material concentration, with or without S9-mix, in any tester strain in either assay. The solvent and positive controls produced the appropriate response in the respective tester strains. Results of the initial and confirmatory mutation assays are summarized in Appendix Tables 1 and 2, respectively (MRID 41651902, pp. 15 and 16).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. Methanearsonic acid was tested to a limit dose of 10,000 µg/plate, proper experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains. Methanearsonic acid was not mutagenic, with or without S9-mix, as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5100[4] (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

[4]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

2. Protocol: The standard assay was performed by adding 100 µL of a tester strain culture and 50 µL of the desired concentration of test material or solvent to 2.5 mL of molten selective top agar at 45 ± 2°C. For assays with metabolic activation, 0.5 mL of S9-mix replaced 0.5 mL of molten selective top agar. After vortexing, the mixture was poured onto the surface of 25 mL of bottom agar. When the overlay solidified, the plate was inverted and incubated for 48 hours at 37 ± 2°C. Selective top agar was 0.8% agar (w/v) and 0.5% NaCl (w/v) supplemented per 100 mL with 10 mL of sterile 0.5 mM L-histidine/0.5 mM D-biotin solution. Minimal bottom agar was Vogel-Bonner minimal medium E containing 1.5% (w/v) agar. Nutrient bottom agar was minimal bottom agar supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2 (dry powder). Revertant colonies for a given tester strain within a given test material dilution series were counted either entirely by hand or entirely by automated colony counter.

A positive response required at least a doubling in the average number of revertants per plate over the solvent control value in strain TA98, TA100 or TA1535 accompanied by a positive dose-response. An increase in the average number of revertants per plate at least three-fold over the solvent control value was required for strains TA1537 and TA1538. An increase between two- and three-fold in these two tester strains must be confirmed by a repeat assay.

II. REPORTED RESULTS

The concentrations of test material used in this study were analytically determined to be as intended (i.e., ≥99.3% of nominal).

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of methanearsonic acid ranging from 10 to 10,000 µg/plate were tested (one plate per dose) in a preliminary cytotoxicity assay using strain TA100. Evidence of cytotoxicity in this assay is a reduction in the number of revertants per plate compared to the solvent control value and/or a thinning or absence of the background lawn of bacteria. No cytotoxicity was seen at any concentration of test material up to and including 6667 µg/plate, with or without S9-mix. Slight thinning of the background lawn was noted at 10,000 µg/plate, with and without S9-mix. Therefore, 10,000 µg/plate was selected as the upper concentration for the mutagenicity assays.

B. MUTAGENICITY ASSAY

Five concentrations of methanearsonic acid ranging from 667 to 10,000 µg/plate were tested with and without S9-mix in two independent mutation assays. All five tester strains were used at each concentration and all plating was in triplicate. There was no evidence of cytotoxicity at any test material concentration, with or without S9-mix, in any tester strain in either assay. Likewise, there was no increase in the number of revertants

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Pages 65 through 66 are not included.

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APPENDIX
(MRID 41651902)

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY

EPA Reviewer: N. McCarroll, Date 7/5/00
Toxicology Branch 1 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D., Date 7/5/00
Reregistration Branch 4 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics (chromosomal aberrations) in Chinese hamster ovary (CHO) cells [OPPTS 870.5375 (§4-2)].

DP BARCODE: D265953
SUBMISSION CODE: S579557
P.C. CODE: 013803
TOX. CHEM. NO.: 582

TEST MATERIAL (PURITY): Methanearsonic acid (99.8% a.i.)

SYNONYMS: T-168-2

CITATION: Chun, J.S. and J.C. Killeen (1989) *In vitro* chromosomal aberration assay in Chinese hamster ovary (CHO) cells with methanearsonic acid (MAA). Microbiological Associates Inc., Division of Genetic Toxicology, 9900 Blackwell Road, Rockville, Maryland 20850. Project ID No.: T8471.337001, October 31, 1989. MRID 41651903. Unpublished.

SPONSOR: Fermenta ASC Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (chromosomal aberrations) (MRID 41651903), Chinese hamster ovary (CHO-K_1) cell cultures were exposed to Methanearsonic acid (Lot No. 107/84, 99.8% a.i.) in distilled water in two independent assays. Concentrations tested in the initial assay were 625, 1250, 2500, 5000 µg/mL, with and without metabolic activation (S9-mix), and in the confirmatory assay were 1250, 2500, 5000 and 10,000 µg/mL, with and without S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Methanearsonic acid was tested up to a slightly cytotoxic concentration, limited by solubility in the solvent, distilled water. In the first cytogenetic assay, microscopic examination of the cell monolayer revealed slight cytotoxicity at 2500 and 5000 µg/mL in the absence of S9-mix and at 5000 µg/mL in the presence of S9-mix but the mitotic index was not reduced. In the second cytogenetic assay, cytotoxicity was seen at 5000 and 10,000 µg/mL in the absence of S9-mix and at 10,000 µg/mL in the presence of S9-mix. The mitotic index at 10,000 µg/mL without S9-mix was 50% of the solvent control value. Solvent and positive controls induced the appropriate response. **There was no evidence that Methanearsonic acid induced a clastogenic response at any dose either in the absence or the presence of S9 activation.**

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DATA EVALUATION REPORT

MONOSODIUM METHANEARSONIC ACID (MSMA)

STUDY TYPE: *IN VITRO* CHROMOSOMAL ABERRATION [OPPTS 870.5375 (§84-2)]
MRID 41651903

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis
Life Sciences Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No.00-36

Primary Reviewer: Bradford L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield JUN 15 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast JUN 15 2000
Robert H. Ross, Group Leader	Signature: Date:	Robert H. Ross JUN 15 2000
Quality Assurance: LeeAnn Wilson, M.A.	Signature: Date:	L. A. Wilson JUN 15 2000

Disclaimer

This Data Evaluation Report may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

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4. Test compound concentrations used

Preliminary cytotoxicity assay:
Nonactivated and activated conditions:
0.5, 1.5, 5, 15, 50, 150, 500, 1510, 5000 µg/mL

Initial cytogenetic assay:
Nonactivated and activated conditions:
625, 1250, 2500, 5000 µg/mL

Confirmatory cytogenetic assay:
Nonactivated and activated conditions:
1250, 2500, 5000, 10,000 µg/mL

5. Test cells

Mammalian cells in culture; Chinese hamster ovary CHO-K_1 cells.

Properly maintained? information not provided.

Cell line or strain periodically checked for Mycoplasma contamination? information not provided.

Cell line or strain periodically checked for karyotype stability? information not provided.

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay

The preliminary cytotoxicity assay evaluated the effects of methanearsonic acid on mitotic index and cell cycle delay. CHO cells were treated with the solvent control and with nine concentrations of the test material ranging from 0.5 to 5000 µg/mL in the presence and absence of S9-mix. The medium used was McCoy's 5A medium supplemented with 10% fetal bovine serum, 100 units penicillin and 100 µg streptomycin/mL and 2 mM L-glutamine. The cells were treated for six hours without S9-mix. Two hours after the start of treatment, 1 mM 5-bromo-2'-deoxyuridine (BrdU) was added to the cultures. Treatment time was two hours in the presence of S9-mix. At the end of the treatment period, the treatment medium was removed, the cells were washed and refed with complete medium containing 0.01 mM BrdU and incubated an additional 22 hours from the start of BrdU treatment. Colcemid (0.1 µg/mL) was added and incubation continued for two more hours. The cells were then harvested and slides prepared, stained and evaluated for effects on cell cycle kinetics. The mitotic index was determined as the number of cells in mitosis per 500 cells.

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This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5375 (§84-2)] for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methanearsonic acid

Description: white crystalline solid
Lot/Batch #: 107/84
Purity: 99.8% a.i.
Stability of compound: stable
CAS #: not provided
Structure: not provided
Solvent used: distilled water
Other comments: none

2. Control materials

Negative: untreated
Solvent/final concentration: distilled water / 50 µL/flask
Positive (concentrations/solvent):
Nonactivation: Triethylenemelamine / 0.5 µg/mL / distilled water
Activation: Cyclophosphamide / 50 µg/mL / distilled water

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none			_ hamster
_ other		_ other	_ other

If other, describe below

S9 mix composition: (in growth medium + 2.5% serum)

NADP	1.4 mg/mL
isocitric acid	2.7 mg/mL
S9-fraction	15 µL/mL

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Fragments seen with an exchange figure were not scored as an aberration but were considered part of an incomplete exchange. Pulverized chromosomes, pulverized cells and severely damaged cells (> 10 aberrations) were also recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The mitotic index was determined as the number of cells in mitosis per 500 cells counted.

The results are considered positive if the number of structural aberrations per cell and the percentage of cells with aberrations are significantly increased over the solvent control value at one or more concentrations with an accompanying dose response. A statistically significant increase at the high dose only with no dose response is considered suspect while a significant increase at one dose only other than the high dose is considered equivocal.

g. Statistical analysis

Data were evaluated for statistical significance at $p \leq 0.05$, using Fisher's exact test.

II. REPORTED RESULTS

An analysis of the stock solutions of test material used in the study confirmed that the concentrations were as intended (i.e., ≥99.3% of nominal).

A. PRELIMINARY CYTOTOXICITY ASSAY

Nine concentrations of methanearsonic acid ranging from 0.5 to 5000 µg/mL were tested, with and without S9-mix, in the preliminary cytotoxicity assay. In the absence of S9-mix, the mitotic index at 24 hours varied in a non-dose-related manner from 76% to 85% of the solvent control value at all concentrations except the highest where the mitotic index was 42% of the control value. The percentage of cells in M_2 at 24 hours ranged from 95% to 99% in a non-dose-related manner compared to the solvent control value of 98%. In the presence of S9-mix, the mitotic index at 24 hours varied in a non-dose-related manner from 79% to 100% of the solvent control value at all concentrations except the highest where the mitotic index was 64% of the control value. The percentage of cells in M_2 at 24 hours ranged from 92% to 99% in a non-dose-related manner compared to the solvent control value of 98%.

B. CYTOGENETIC ASSAY

Methanearsonic acid concentrations of 625, 1250, 2500 and 5000 µg/mL were tested, with and without S9-mix, in the first cytogenetic assay using duplicate cultures at each dose. The upper dose was based on the solubility of the test material in distilled water. Microscopic examination of the cell monolayer revealed slight cytotoxicity at 2500 and 5000 µg/mL in the absence of S9-mix and at 5000 µg/mL in the presence of S9-mix. The

2. Cytogenetic assay

a. Cell treatment

Cells exposed to test compound, solvent, or positive control for 8 hours (nonactivated), 2 hours (activated)

b. Spindle inhibition

Inhibition used/concentration: Colcemid / 0.1 µg/mL

Administration time: 2 hours (before cell harvest)

c. Cell harvest

Cells exposed to test material, solvent or positive control were harvested 2 hours after termination of treatment (nonactivated), 8 hours after termination of treatment (activated)

d. Details of slide preparation

Following colcemid treatment, the cells were harvested by trypsinization and collected by centrifugation. Cells were swollen with 0.075 M KCl, washed with two changes of fixative (methanol:acetic acid, 3:1 v/v) and stored in fixative overnight or longer at 4 ± 2°C. Slides were prepared by collecting the cells by centrifugation, resuspending them in fresh fixative and placing one or two drops of the cell suspension on glass microscope slides. After air-drying, the slides were stained with 5% Giemsa and permanently mounted.

e. Metaphase analysis

No. of cells examined per dose: 100 (50 per duplicate culture)

Scored for structural: Y

Scored for numerical: N

Coded prior to analysis: Y

f. Evaluation criteria

Cells with 20 ± 2 centromeres were evaluated for chromatid and isochromatid breaks and exchange figures (quadriradials, triradials and complex rearrangements) and for chromosome breaks and exchange figures (dicentrics and rings). Fragments seen in the absence of an exchange figure were scored as breaks.

APPENDIX
(MRID 41651903)

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mitotic index was not reduced relative to the solvent control at any test material concentration. There was no statistically significant increase in the number of aberrations per cell or in the percentage of cells with aberrations over the solvent control values, with or without S9-mix, at any tested methanearsonic acid concentration. Solvent and positive control values were appropriate. Results of the first assay are summarized in Appendix Table 1 (MRID 416519003, p. 16).

In the second (confirmatory) cytogenetic assay, methanearsonic acid concentrations of 1250, 2500, 5000 and 10,000 µg/mL were tested. Microscopic examination of the cell monolayer revealed cytotoxicity at 5000 and 10,000 µg/mL in the absence of S9-mix and at 10,000 µg/mL in the presence of S9-mix. The mitotic index at 10,000 µg/mL without S9-mix was 50% of the solvent control value. There was no statistically significant increase in the number of aberrations per cell or in the percentage of cells with aberrations over the solvent control values, with or without S9-mix, at any tested methanearsonic acid concentration in the second assay. Solvent and positive control values were appropriate. Results of the second assay are summarized in Appendix Table 2 (MRID 41651903, p. 17).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. Methanearsonic acid was tested to a sufficiently high concentration, proper experimental protocol was followed and the solvent and positive control values were appropriate. There was no evidence that methanearsonic acid increased the frequency of chromosomal aberrations as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5375 (§84-2)] for *in vitro* cytogenetic mutagenicity data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

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DATA EVALUATION REPORT 014343

(7)

MONOSODIUM METHANEARSONIC ACID (MSMA)

STUDY TYPE: MAMMALIAN CELLS IN CULTURE GENE MUTATION ASSAY IN L5178Y TK$^{+/-}$ MOUSE LYMPHOMA CELLS [OPPTS 870.5300 (§84-2)]

MRID 41651904

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-36

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Quality Assurance:
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Date: JUN 15 2000

Disclaimer

This review may have been altered subsequent to the contractor's signature above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

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_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

750 µg/mL in the confirmatory assay. Higher concentrations in both assays were too toxic to clone. The responses induced by solvent and positive control were appropriate. **There was no evidence that Methanearsonic acid induced a mutagenic response either in the presence or the absence of S9 activation.**

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5300 (§84-2)] for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methanearsonic acid

   Description: white crystalline solid
   Lot/Batch #: 107/84
   Purity: 99.8 % a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: deionized water
   Other comments: none

2. Control materials

   Negative: none
   Solvent/final concentration: deionized water
   Positive (concentrations/solvent):
   Nonactivation: Ethyl methanesulfonate / 0.25, 0.5 µg/mL / DMSO
   Activation: 7,12-Dimethylbenz(a)anthracene / 2.5, 5.0 µg/mL / DMSO

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none		_ hamster	_ other
_ other			_ other

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EPA Reviewer: N. McCarroll, Date 7/6/00
Toxicology Branch 1: (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D., Date 7/6/00
Reregistration Branch 4 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in L5178Y TK$^{+/-}$ mouse lymphoma cells [OPPTS 870.5300 (§84-2)]

DP BARCODE: D265953
P.C. CODE: 013803

SUBMISSION CODE: S579557
TOX. CHEM. NO.: 582

TEST MATERIAL (PURITY): Methanearsonic acid (MAA) (Monosodium methanearsonic acid (MSMA), 99.8% a.i.)

SYNONYMS: T-168-2

CITATION: Chun, J.S. and J.C. Killeen (1989) L5178Y TK$^{+/-}$ mouse lymphoma mutagenesis assay with methanearsonic acid. Microbiological Associates Inc., Division of Genetic Toxicology, 9900 Blackwell Road, Rockville, Maryland 20850. Project ID No.: T8471.701020, December 7, 1989. MRID 41651904. Unpublished

SPONSOR: Fermenta ASC Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the TK locus (MRID 41651904), L5178Y TK$^{+/-}$ mouse lymphoma cells cultured *in vitro* were exposed to methanearsonic acid (Lot No. 107/84, 99.8% a.i..) in deionized water at concentrations of 300, 400, 534, 712, 949, 1266, 1688, 2250, 3000 and 4000 µg/mL in the absence of mammalian metabolic activation (S9-mix) and at concentrations of 71, 95, 127, 169, 225, 300, 400, 534, 712, 949, 1266 and 1688 µg/mL in the presence of S9-mix. A confirmatory assay was conducted at concentrations of 2000, 3000, 4000, 5000 and 6000 µg/mL without S9-mix and at concentrations of 200, 350, 500, 650, 750, 850 and 950 µg/mL in the presence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Methanearsonic acid was tested up to cytotoxic concentrations. The high dose of 4000 µg/mL was based on the solubility limit of the test material in the dosing solution. In the mutation assays without S9-mix, the percent total growth decreased with increasing dose, reaching 48% of the solvent control value at 4000 µg/mL in the initial assay and 20% and 26% of the solvent control value in duplicate cultures at 4000 µg/mL in the confirmatory assay. Concentrations of 5000 and 6000 µg/mL were too toxic to clone in the confirmatory assay. In the mutation assays with S9-mix, the percent total growth decreased with increasing dose, reaching 14% of the solvent control value at 949 µg/mL in the initial assay and 10% of the solvent control value at

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Activated conditions:
71, 95, 127, 169, 225, 300, 400, 534, 712, 949, 1266 and 1688 µg/mL

Confirmatory mutation assay:
Nonactivated conditions:
2000, 3000, 4000, 5000 and 6000 µg/mL
Activated conditions:
200, 350, 500, 650, 750, 850 and 950 µg/mL

B. TEST PERFORMANCE

1. Cell treatment:

a.. Cells exposed to test compound, negative/solvent or positive controls for: 4 hours (nonactivated) 4 hours (activated)

b. After washing, cells cultured for 2 days (expression period) before cell selection:

c. After expression, 1×10^6 cells/dish (3 dishes/ group) were cultured for 10 - 12 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 10 - 12 days without selective agent to determine cloning efficiency.

2. Statistical methods

Statistical significance was determined by Kastenbaum and Bowman Tables ($p \leq 0.05$).

3. Evaluation criteria

The number of colonies per plate (both TFT selection plates and viable count plates) were counted using an automatic colony counter whenever possible or counted manually otherwise. Three counts per plate were made and the median count recorded. The mutant frequency was reported as the number of mutants per 10^4 surviving cells. The results were considered positive if there was a positive dose response and one or more doses in the 10% or greater total growth range induced a mutant frequency at least twice that of the solvent control. A positive result in the initial assay must be reproduced in a confirmatory assay. The results were considered equivocal if one or more of the three highest doses with 10% or greater total growth showed a two-fold or greater increase in mutant frequency over the solvent control in the absence of a dose-response. The results were also considered equivocal if there

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S9 mix composition:

Isocitric acid	11.25 mg/mL
NADP	6.0 mg/mL
F_0P^*	0.75 mL/mL
S-9-homogenate	0.25 mL/mL

* Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronic

4. Test cells: mammalian cells in culture

x mouse lymphoma L5178Y cells
_ Chinese hamster ovary (CHO) cells
_ V79 cells (Chinese hamster lung fibroblasts)

Properly maintained? Y
Periodically checked for Mycoplasma contamination? information not provided
Periodically checked for karyotype stability? information not provided
Periodically "cleansed" against high spontaneous background? Y

Media: F_0P - Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronic;
$F_{10}P$ - F_0P with 10% heat inactivated horse serum

5. Locus examined

x thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
2 - 4 µg/mL trifluorothymidine (TFT)

_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)
_ Na^+/K^+ ATPase
Selection agent: _____ ouabain

6. Test compound concentrations used

Preliminary cytotoxicity test:
Nonactivated and activated conditions:
0.1, 1, 10, 100, 1000, 4000 µg/mL

Initial mutation assay:
Nonactivated conditions:
300, 400, 534, 712, 949, 1266, 1688, 2250, 3000 and 4000 µg/mL

surviving cells. A colony size analysis was done but, according to the study authors, the small number of colonies on both the solvent control and treated cultures precluded any definitive conclusions about the biological significance of the positive result. The results of the initial assay were considered equivocal. In the confirmatory assay, a statistically significant increase in mutant frequency over the control value of 0.7 mutants per 10^4 surviving cells was seen at 750 µg/mL (1.0 mutants per 10^4 surviving cells) but did not meet the two-fold increase requirement for a positive response. The second culture at 750 µg/mL was lost. The results of the confirmatory assay were considered negative. Solvent and positive control values were appropriate. Results of the initial and confirmatory mutation assays with S9-mix are summarized in Appendix Tables 4 and 5, respectively (MRID 41651904, pp. 19 and 20).

The overall conclusion based on the two studies was no biologically significant increase in mutant frequency due to methanearsonic acid treatment.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. Methanearsonic acid was tested to sufficiently high concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate. Although the statistically significant increases in mutant frequency seen at the high doses with S9-mix are suggestive of a mutagenic effect, the actual values are low and within the historical solvent control range and are unlikely to be biologically significant.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5300 (§84-2)] for *in vitro* mutagenicity (mammalian forward gene mutation) data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

F3

was a dose response but no culture showed a two-fold or greater increase in mutant frequency over the solvent control value.

II. REPORTED RESULTS

The concentrations of test material used in this study were analytically determined to be as intended (i.e., ≥99.3% of nominal).

A. PRELIMINARY CYTOTOXICITY ASSAY

Six concentrations of methanearsonic acid ranging from 0.1 to 4000 µg/mL were tested in the preliminary cytotoxicity assay. The high dose of 4000 µg/mL was based on the solubility limit of the test material in the dosing solution. Suspension growth was reduced to 35% of the solvent control value at 4000 µg/mL in the absence of S9-mix and to 0% in the presence of S9-mix. The second highest dose, 1000 µg/mL, reduced suspension growth to 79% of the solvent control value in the absence of S9-mix and to 26% of the solvent control value in the presence of S9-mix. Results of the cytotoxicity assay are presented in Appendix Table 1 (MRID 41651904, p. 74).

B. MUTAGENICITY ASSAY

In the absence of S9-mix, ten methanearsonic acid concentrations ranging from 300 to 4000 µg/mL were tested in the initial mutation assay and five concentrations ranging from 2000 to 6000 µg/mL were tested in the confirmatory assay. The percent total growth decreased with increasing dose, reaching 48% of the solvent control value at 4000 µg/mL in the initial assay and 20% and 26% of the solvent control value in duplicate cultures at 4000 µg/mL in the confirmatory assay. Concentrations of 5000 and 6000 µg/mL were too toxic to clone in the confirmatory assay. There was no increase in the mutant frequency at any test material concentration in either assay in the absence of S9-mix. The solvent control values of 1 per 10^4 surviving cells in the initial assay and 0.6 per 10^4 surviving cells in the confirmatory assay are acceptable. Results of the initial and confirmatory mutation assays without S9-mix are summarized in Appendix Tables 2 and 3, respectively (MRID 41651904, pp. 17 and 18).

In the presence of S9-mix, 12 methanearsonic acid concentrations ranging from 71 to 1688 µg/mL were tested in the initial mutation assay and seven concentrations ranging from 200 to 950 µg/mL were tested in the confirmatory assay. The percent total growth decreased with increasing dose, reaching 14% of the solvent control value at 949 µg/mL in the initial assay and 10% of the solvent control value at 750 µg/mL in the confirmatory assay. Higher concentrations in both assays were too toxic to clone. In the initial assay, statistically significant increases in mutant frequency over the solvent control value of 0.4 mutants per 10^4 surviving cells were seen at 712 µg/mL (0.6 mutants per 10^4 surviving cells) and 949 µg/mL (0.8 mutants per 10^4 surviving cells). The latter value met the criterion of a two-fold increase required for a positive response but was within the testing laboratory's historical solvent control range of 0.2 to 1.0 mutants per 10^4

APPENDIX
(MRID 41651904)

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APPENDIX
(MRID 41651904)

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014343

DATA EVALUATION REPORT

MONOSODIUM METHANEARSONIC ACID (MSMA)

STUDY TYPE: OTHER GENOTOXICITY: UNSCHEDULED DNA SYNTHESIS IN PRIMARY RAT HEPATOCYTES/MAMMALIAN CELL CULTURES; [OPPTS 870.5550 (§84-2)]
MRID 41651905

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-36

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Date: JUN 19 2000

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Date: JUN 19 2000

Signature: Robert H. Ross
Date: JUN 19 2000

Signature: L. A. Wilson
Date: JUN 19 2000

Disclaimer

This review may have been altered subsequent to the contractor's signature above.

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_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methanearsonic acid

   Description: white crystalline solid
   Lot/Batch #: 107/84
   Purity: 99.8 % a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: deionized distilled water
   Other comments: none

2. Control materials

   Negative: none
   Solvent/final concentration: deionized distilled water /
   Positive (concentrations/solvent): 7,12-Dimethylbenzanthracene / 3.0 and 5.0 µg/mL (initial assay); 3.0 and 10 µg/mL (confirmatory assay) / DMSO

3. Test compound concentrations used:

   Preliminary cytotoxicity assay:
   0.06, 0.2, 0.6, 2.0, 6.0, 20, 60, 200, 600, 2000 µg/mL
   UDS assays (initial and confirmatory assays) 10, 50, 100, 500, 750, 1000 µg/mL

4. Media

   Plating medium - Williams Medium E (WME) buffered with 0.01 M HEPES, adjusted to pH 7.3 and supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 50 µg gentamycin/mL.

   Perfusion (collagenase) solution - Serum-free WME with HEPES, L-glutamine and gentamycin as in the plating medium and containing 80 - 100 units of collagenase (Type I)/mL.

   Treatment medium - Serum-free WME plus HEPES, L-glutamine and gentamycin as in the plating medium and containing 10 µCi ^{3}H-TdR/mL.

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EPA Reviewer: N. McCarroll
Toxicology Branch 1 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D.
Reregistration Branch 4 (7509C)

Nancy E. McCarroll, Date 7/6/00

Sanjiv Diwan, Date 7/6/00

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures; [OPPTS 870.5550 (§84-2)]

DP BARCODE: D265953
P.C. CODE: 013803

SUBMISSION CODE: S579557
TOX. CHEM. NO.: 582

TEST MATERIAL (PURITY): Methanearsonic acid (MAA) (Monosodium methanearsonic acid (MSMA), 99.8% a.i.)

SYNONYMS: T168-2

CITATION: Chun, J.S. and J.C. Killeen (1989) Unscheduled DNA synthesis assay in rat primary hepatocytes with methanearsonic acid (MAA). Microbiological Associates Inc., Division of Genetic Toxicology, 9900 Blackwell Road, Rockville, Maryland 20850. Project ID No.: T8471.380009, October 31, 1989. MRID 41651905. Unpublished.

SPONSOR: Fermenta ASC Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID 41651905), primary rat hepatocyte cultures were exposed to Methanearsonic acid (Lot No. 107/84, 99.8% a.i.) in deionized distilled water at concentrations of 10, 50, 100, 500, 750 and 1000 µg/mL for 18 to 20 hours in an initial and a confirmatory assay. The autoradiographic procedure was used.

Methanearsonic acid was tested up to cytotoxic concentrations (≥ 600 µg/mL). The responses induced by solvent and positive control values (including DMSO, the positive control solvent) were appropriate. **There was no evidence that Methanearsonic acid induced unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts].**

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5550 (§84-2)] for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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from the three cytotoxicity plates were harvested and the LDH activity in the culture medium determined.

b. Preparation of autoradiographs/grain development

Cells were washed in serum-free WME, swollen in 1% sodium citrate solution and fixed in three 15-minute exchanges of ethanol-glacial acetic acid fixative. Coverslips were allowed to dry for at least one hour and then mounted cell side up on glass slides. The slides were dipped in NTB-2 emulsion at 43 - 45°C, dried for at least 1.5 hours at room temperature and stored in a refrigerator for seven days in light-tight boxes with desiccant. Slides were then developed in Kodak D-19 developer, fixed in Kodak fixer and stained in hematoxylin-sodium acetate-eosin stain and permanently mounted.

c. Grain counting

Slides were coded prior to evaluation. Grain counts from 50 cells per slide (150 cells total per treatment) were made with an automated colony counter. The nuclear grain count and the cytoplasmic grain count (the average grain count of three nuclear-size areas of cytoplasm adjacent to the nucleus) were determined and the net nuclear grain count calculated by subtracting the cytoplasmic count from the nuclear count. The mean net nuclear grain count for each treatment was also calculated and the percentage of cells in repair was recorded.

d. Evaluation criteria

The results for a particular dose level were considered significant if the mean net nuclear grain count was increased by at least five counts compared to the solvent control. The test material was considered positive for UDS induction if there was a positive dose-response and at least one dose produced a significant increase in the mean net nuclear grain count. The results were also considered positive if there was a significant increase in the mean net nuclear grain count in at least two successive doses in the absence of an overall positive dose-response. The results were considered equivocal if there was a significant increase at one dose level in the absence of a positive dose-response. For the test to be valid, the solvent control must have a net nuclear grain count < 1 and the percentage of cells in repair in the negative media control must be < 15%.

e. Statistical analysis

No statistical analysis was performed. Results were considered significant if the mean net nuclear grain count for a particular dose level was increased at least five counts over the solvent control value.

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5. Test cells

Mammalian cells in culture/primary rat hepatocytes. Primary rat hepatocytes from healthy male Sprague-Dawley rats.

6. Cell preparation:

a. Perfusion technique

Rats were killed by inhalation of metofane and the liver perfused *in situ* through the hepatic portal vein with 0.5 mM EGTA in Hanks' Balanced Salt Solution (pH 7.3) at 8 mL/minute for 1 - 2 minutes. The inferior vena cava was clamped off and the thoracic vena cava cannulated through the heart or the heart was punctured and the perfusion rate then increased to 20 - 40 mL/minute. After perfusion of about 120 mL of EGTA solution, 250 mL of collagenase solution was perfused through the liver at 20 - 40 mL/minute. The liver was then removed and the capsule opened.

b. Hepatocyte harvest/culture preparation

The excised liver was shaken in collagenase solution and the cells removed by gentle combing of the liver lobes with a stainless steel comb or by passing the cells through a stainless steel sieve. Cells were pooled, counted and approximately 5×10^5 cells seeded into preconditioned 35 mm tissue culture plates containing coverslips for the UDS assay or without coverslips for the cytotoxicity test. Three plates per dose were used for cytotoxicity determination and three plates per dose used for the UDS assay.

B. TEST PERFORMANCE

1. Cytotoxicity assay

Hepatocyte cultures were treated with methanearsonic acid 90 - 180 minutes after seeding. After 18 - 20 hours of incubation, lactic acid dehydrogenase activity in the medium was determined as a measure of cytotoxicity.

2. UDS assay

a. Treatment

Three replicate plates were treated with the desired concentration of test material, positive control or solvent control and 10 µCi/mL of ^{3}H-TdR was added to each plate. Three additional plates were used at each experimental point for cytotoxicity determination. Treatment continued for 18 - 20 hours at which time the cells

APPENDIX

(MRID 41651905)

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II. REPORTED RESULTS

The concentrations of test material used in the study were analytically determined to be as intended (i.e., ≥99.3% of nominal).

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of methanearsonic acid ranging from 0.06 to 2000 µg/mL were tested in the preliminary cytotoxicity assay. No cytotoxicity, as based on LDH release relative to the solvent control, was seen at any dose up to and including 600 µg/mL (the second highest concentration tested). Cytotoxicity was apparent at 2000 µg/mL where the relative survival was 6%. Morphological examination of the hepatocytes showed moderate cytotoxicity at 2000 µg/mL and slight cytotoxicity at 600 µg/mL. An acidic shift in pH was seen on initiation of treatment at 2000 and 600 µg/mL and also at the termination of treatment at 2000 µg/mL. Based on cytotoxicity considerations, 1000 µg/mL was selected as the upper dose for the UDS assays.

B. UDS ASSAY

Six concentrations of methanearsonic acid ranging from 10 to 1000 µg/mL were tested in both the initial and confirmatory UDS assays. Fifty cells per slide (150 cells per group) were evaluated for UDS. Cultures treated at 1000 µg/mL were not evaluated for UDS activity in either assay due to excessive cytotoxicity seen on examination of the fixed and stained cells. The cell morphology at all lower concentrations appeared normal. The excessive cytotoxicity at 1000 µg/mL was not reflected in the LDH release as the relative survival at 1000 µg/mL, based on relative LDH release of treated and solvent control cultures was 77% in the initial assay and 80% in the confirmatory assay, not much different than the 81 % and 87% relative survival seen at 750 µg/mL in the initial and confirmatory assays, respectively. An acidic pH change, as detected by an increased yellow color, was seen at the initiation of treatment at 1000 µg/mL and possibly affected cytotoxicity.

There was no increase in the mean net nuclear grain count at any of the five concentrations of test material in either assay over the solvent control values of -1.1 ± 2.4 in the initial assay and 0.3 ± 2.2 in the confirmatory assay. The solvent and positive control values (including DMSO, the positive control solvent) were appropriate. Results of the initial and confirmatory UDS assays are summarized in Appendix Tables 1 and 2, respectively (MRID 41651905, pp. 15 and 16).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. Methanearsonic acid was tested to a sufficiently high concentration, proper experimental protocol was followed and the solvent and positive control values were appropriate. There was no evidence that methanearsonic acid induced UDS over the solvent control level as tested in this study.

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EXECUTIVE SUMMARY

014343 (9)

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: COMBINED CHRONIC TOXICITY/ONCOGENICITY-RAT
[OPPTS 870.4300 (§83-5)]
MRID NO. 41669001

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross for D.L. Fefee
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: H.T. Borges
Date: MAY 22 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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Page_____ is not included in this copy.

Pages 98 through 99 are not included.

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Supplement to Tox Document 009478, review for Accession 41669001, 104-week combined chronic oral toxicity/oncogenicity study-rat. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 9/7/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 9/12/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 104-Week Combined Chronic Toxicity/Oncogenicity - Rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D265953 SUBMISSION CODE: S579557
PC CODE: 013803 TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (purity 98.42-98.80%)

SYNONYM: Monosodium acid methanearsonate, MSMA

CITATION: Crown, S., A. Nyske, T. Waner (1990) Methanearsonic acid - combined chronic feeding and oncogenicity study in the rat. Life Science Research Israel, Ltd., P.O. Box 139, Ness Ziona 70 451 Israel. Laboratory Report No. PAL/004/MAA, July 18, 1990. MRID 41669001. Unpublished.

SPONSOR: Luxembourg Industries (Pamol), Ltd., 27 Hamered St., P.O. 13, Tel-Aviv 61100, Israel.

EXECUTIVE SUMMARY:

In a combined chronic toxicity/carcinogenicity feeding study (MRID 41669001), methanearsonic acid (purity 98.42-98.80%; Batch No. 107/84) was administered in the diet to 60 Fischer F344 rats/sex/dose at dose levels of 0, 50, 400 and 800-1300 ppm (0, 3.2, 27.2, and 93.1 mg/kg/day for males and 0, 3.8, 32.9, and 101.4 mg/kg/day for females) for 104 weeks. The high-dose group of 60 animals/sex received 1300 ppm until week 53. Because of excessive mortality (32% of males), the highest dose was reduced to 1000 ppm until week 60 and to 800 ppm for the remainder of the study. At termination, the cumulative mortality was 42, 50, 45, and 67% of males and 20, 33, 22, and 35% of females for the 0, 50, 400, and 800 ppm groups, respectively. The following were measured during the study: clinical signs, body weight, food consumption, water consumption, opthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, neoplastic and non-neoplastic histopathology.

Beginning at week 4-5, diarrhea was observed in all rats at the highest dose level and 27/60 males and 45/60 females of the 400 ppm group. Body weights were statistically decreased from week 7 through termination for males of the 400 ppm groups and from week 4 through termination for high-dose males. Body weights were statistically decreased from week 54

EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: COMBINED CHRONIC TOXICITY/ONCOGENICITY-RAT
[OPPTS 870.4300 (§83-5)]
MRID NO. 41669001

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M. Signature:_____
Date: _____

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

The chronic LOAEL was 400 ppm (27.2 mg/kg/day for males and 32.9 mg/kg/day for females) based on decreased body weights, body weight gains, food consumption, histopathology of gastrointestinal tract, thryoid, and increased incidence of parathyroid adenomas.

The chronic NOAEL was 50 ppm (3.2 mg/kg/day for males and 3.8 mg/kg/day for females).

This study is classified **Acceptable/Guideline** and satisfies the guideline requirements for a combined chronic toxicity/oncogenicity study in rats [OPPTS 870.4300 (§83-5)].

through termination for females of the 400 ppm groups and from week 4 through termination for high-dose females. Overall, week 0-104 weight gains were decreased for the 400 ppm (-11% M and -22% F) and high- dose groups (-22% M and -34% F).

Total protein, albumin, cholesterol, and calcium, concentrations were statistically decreased in male and female rats in the highest dose group, results consistent with inanition. Other sporadic statistically changes in clinical chemistry parameters were found, but were of not of biological or toxicological significance. No remarkable hematological effects were found.

Starting at approximately week 7, food consumption by the high-dose male and female groups was increased compared to control (+37% M and +15% F). Beginning at week 1 for the high-dose females and week 7 for the 400 ppm and high-dose males and 400 ppm females. Throughout the study, water consumption was increased 29% and 31% for males and females of the 400 ppm group and 149% and 108% in males and females of the high-dose group. Urine volume was statistically decreased with a parallel increase in specific gravity in high-dose males and females throughout the study. In females of the 400 ppm group, a decrease in urine volume and increased specific gravity were observed at 12 and 18 months. Urine pH was decreased in males throughout the study in the high-dose.

Absolute kidney weights were statistically increased in females of the 400 ppm group; relative kidney and liver weights were statistically increased in 400 ppm and high-dose females. Gross pathology findings from animals that died or were sacrificed moribund included emaciation and dehydration, reduced abdominal fat pads, along with thickened walls, and edematous, congested, hemorrhagic, necrotic, ulcerated, or perforated stomach, small intestine and/or large intestine, with secondary lesions in adjacent organs including the prostate, testes, kidneys, urinary bladder, epididymides, seminal vesicles, and ureters.

Histopathology findings, including acute inflammation, mucosal congestion, inflammation and ulceration or perforation of the cecum, colon, and rectum, with evidence of acute or chronic peritonitis, were observed mainly in the high-dose groups and sporadically in the 400 ppm groups and indicated that the large intestine was the primary target for the irritant effect of the test material. Ureteral damage occurring as a sequella to intestinal perforation resulted in severe kidney pathology, including hydronephrosis, cortical tubular cystic dilatation, pyelonephritis, papillary necrosis, and glomerulonephropathy.

At 6 months, a dose dependant decrease in T3 with a parallel decrease in T4 was observed in high-dose males. Females exhibited an increase in T4 (no change in T3) at 12 months in the 400 ppm group and at 12 and 18 months in the high-dose group. Increase in height of the thyroid follicular epithelium was observed at the 400 ppm and high-dose levels of both sexes.

Increased incidences of parathyroid adenomas with a positive dose-related trend were observed in both sexes (males: 1/52, 0/49, 4/53, and 4/45 for the control, 50, 400 ppm and high-dose groups, respectively; females: 0/46, 0/44, 0/40, and 4/45 for control, 50 ppm, 400 ppm, and high-dose groups, respectively), although statistical significance was not attained with respect to either sex. The incidence in the present study was outside the incidence range of historical controls given in the study report. Dosing was considered adequate for carcinogenicity testing.

A suggestion of an increased incident of parathyroid gland adenomas was seen in males at the intermediate and high levels and in the females at the high level.

Mortality was increased in the high level animals when compared to the respective controls.

Reduced body weight gain occurred in males at the high level and in females at both the intermediate and high levels.

High dose animals showed acute inflammation, mucosal ulceration and perforations of the large intestine (cecum, colon and rectum). The abdominal wall showed evidence of acute or chronic peritonitis. The intermediate dose was similarly but sporadically affected.

Maximum tolerated dose (MTD) = intermediate dose. The NOEL = 3.2 mg/kg/day (males), 3.8 mg/kg/day (females). LOEL (systemic toxicity) = 27 mg/kg/day (males), 33 mg/kg/day (females).

Recommendations:

This study should be referred to the RFD Mini Peer Review for consideration because of the carcinogenic response in the parathyroid gland as evidenced by adenomas seen in males at the intermediate and high dose and in females at the high dose.

014343

CASWELL FILE

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

009175

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MAY 1 1992

MEMORANDUM:

Subject: Review of Toxicology Studies with Methanearsonic Acid/Methanearsonic acid, monosodium salt to support reregistration of the test substance. (Toxchem Number 582, HED Project No. 1-1668, Barcode number: D165492)

FROM: Steven L. Malish, Ph.D., Toxicologist S. L. Malish 4/15/92
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Barbara Briscoe PM (51)/Betty Crompton PM Team Reviewer
Special Review and Reregistration Division
HED (H7508W)

THRU: Elizabeth Doyle, Ph.D., Section Head E. A. Doyle 4/15/92
Tox. Section II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 4/25/92
Tox. Branch II
HED (H7509C)

ACTION REQUESTED: Review of toxicology studies for reregistration requirements.

Study Summarized

MRID 416690-01, Combined Chronic Toxicity/Oncogenicity Study - rats (83-5); Core - guideline

Methanearsonic acid was incorporated into the diet of 4 groups of 60 Fischer F344 rats per sex at concentrations of 0, 3.2, 27 and 93 mg/kg/day (males) and 0, 3.8, 3.3 and 101 mg/kg/day ppm (females) for 104 weeks.

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The maximum tolerated dose (MTD) based on a decreased body weight gain for chronic dietary administration of MAA was 400 ppm.

A suggestion of an increased incident of parathyroid gland adenomas was seen in males at the intermediate and high levels and in the females at the high level.

The NOEL = 50 ppm (low level); the LOEL (systemic toxicity) = 400 ppm (intermediate level).

CLASSIFICATION:

Core: guideline

The study satisfies the guideline requirement (83-5) for a combined chronic toxicity/oncogenicity study.

A. MATERIALS:

QUALITY ASSURANCE:

The study and final document and addendum(s) were inspected and reviewed by the Quality Assurance Group under the general requirements of the Good Laboratory Practices (December 22, 1978). The quality assurance document was signed by the study director, submitter/sponsor and the manager of the quality assurance unit.

1. Test Compound:

Chemical:	methanearsonic acid
Trade Name:	MAA
Batch No.	107/84
Purity:	> 99.8% (Label); 98.42 - 98.80% (Lab Analysis)
CAS:	124-58-3
Description:	white crystals
Storage:	room temperature

a. Analysis of Formulated Diets:

Stability

Stability was determined from the trial mix prepared prior to commencement of the study. Two samples from each concentration were analyzed after 16 days of storage. The material was within -4 to 6% of the required concentration during the 16 day period (Table 1).

009478

Reviewed by Steven L. Malish, Ph.D. Steven J. Malish 12/10/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 4/15/92
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE:	Combined Chronic Toxicity/Oncogenicity Study (83-5)
MRID NO:	416690-01
TEST MATERIAL:	Methanearsonic Acid
SYNONYMS:	MAA
SPONSOR:	Luxembourg Industries (Pamol) Ltd. 27 Hamered St. P.O. 13 Tel-Aviv 61000, Israel
TESTING FACILITY:	Life Science Research Israel, Ltd. PO Box 139, Ness Ziona, 70 451 Israel
LAB STUDY NO.:	PAL/004/MAA
TITLE OF REPORT:	Methanearsonic Acid Combined Chronic Feeding and Oncogenicity Study In The Rat
AUTHORS:	S. Crown, A. Nyske, T. Waner
REPORT ISSUED:	July 18, 1990

CONCLUSIONS:

Methanearsonic acid (MAA) was incorporated into the diet of 4 groups of 60 Fischer F344 rats per sex at concentrations of 0 (Control), 50, 400 or 1300 ppm for 104 weeks. The 1300 ppm concentration was reduced to 1000 ppm during week 53 and to 800 ppm at week 60 because of excessive mortality.

Mortality was increased in the high level animals when compared to the respective controls.

Reduced body weight gain occurred in males at the high level and in females at both the intermediate and high levels.

High level animals had acute inflammation, mucosal ulceration and perforations of the large intestine (cecum, colon and rectum). The abdominal wall showed evidence of acute or chronic peritonitis. The intermediate dose was similarly but sporadically affected. 3

A reduction in the thyroid weight of the intermediate and high level females and the intermediate level males was observed together with an increase height of the thyroid follicular epithelium of the high and intermediate levels of both sexes.

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63, 68 and 73 of the study. All results were positive for methanearsonic acid.

2. Test Animals:

Species: Rats
Strain: Fischer F344
Age: 4 to 5 weeks of age upon receipt
Weight: Males 70-134 gm; Females 73-112 gm at start of study
Source: Charles River Breeding Laboratories, Margate, Kent, England

B. STUDY DESIGN:

1. Animal Assignments:

Sixty (60) animals per sex were assigned randomly to four (4) test groups and administered 0 ppm (group 1), 50 ppm (group 2), 400 ppm (group 3) and 1300 ppm (group 4) ad-mixed in the feed. The high dose concentration was subsequently reduced to 1000 ppm at week 53 and to 800 ppm at week 60 because of excessive mortality (Table 3).

Reduction of treatment levels were made following discussions with William Burnam of the USEPA/OPP in 1986.

Table 3

Animal Test Group Assignments [1]

Group	Treatment	Dietary Level (ppm)	Animals on Test (M/F)
1	Control	0	60/60
2	MAA	50	60/60
3	MAA	400	60/60
4	MAA	1300[2]	60/60

[1] Adapted from original report Vol 1, p. 29.
[2] Reduced to 1000 ppm at week 53; reduced again to 800 ppm at week 60.

2. Diet:

Animals received the basal diet of Altromin 1321N chow and water ad libitum.

3. Diet preparation:

Methanearsonic acid was incorporated into the powdered basal diet at the appropriate levels for the test diets each week. An initial premix was followed by dilution with further quantities of the diet and mixed for 10-15 minutes in a horizontal mixer.

Table 1

Stability of MMA 16 days After Admix to the Feed[1]

Group	No. of Samples	Concentration (ppm) Required	Achieved (%[2])
1	-	-	- -
2	2	50	52 (+4%)
3	2	400	385 (-4%)
4	2	1300	1380 (+6%)

[1]Adapted from original report, Vol. 9, p. 1731 thru 1740.
[2]Percent difference between achieved and required.

Homogeneity

Homogeneity of MAA dispersal in the rodent diet was initially determined from a trial mix prepared prior to commencement of the study. The mixture was sampled from 6 different spots of each concentration and analyzed for MAA. The same procedure was adopted with samples from trial mixes prepared during weeks 41, 53 and 60.

The percentage change in the standard deviation during the 104 week study was within ±7% of the mean values.

Table 2

Homogeneity of MAA in Rodent Diet at Various Time Intervals[1]

Time (Weeks)	Concentration (ppm) 0	50	400	1300
0	-	48±5^	401±13	1300±28
4	-	51±3	432±14	1177±67
53[a]	-	51±2	407±16	1014±27
60[b]	-	45±8	423±3	827±4

[1]Adapted from the original report, Vol. 9, p. 1731 - 1740.
^ standard deviation
[a]High level concentration decreased from 1300 ppm to 1000 ppm.
[b]High concentration decreased from 1000 ppm to 800 ppm.

Quantitative Analysis

Spot checks were made to verify the test material content in the 22 time periods during the 104 week study. Samples were taken at each concentration level.

As the quantitative analytical technique was not specific for MAA, qualitative chromatographic analysis were made during weeks 61, 62,

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2. Mortality

Severely debilitated animals were sacrificed or isolated to prevent cannibalism. Animals judged in extremis were sacrificed to preclude autolysis.

Animals found dead outside normal working hours were preserved at 4° C and necropsied as soon as possible the following day. A complete necropsy was performed in all cases.

At termination, the percentage mortality in males was 42, 50, 45 and 67% in Groups 1 thru 4, respectively, and 20, 33, 22, and 35% for females in Groups 1 thru 4, respectively (Table 4).

Table 4

Cumulative and Percentage Mortality at Various Time Periods During the 104 Week Study[1]

Week	1M	2M	3M	4M
	Males*			
14	0(0%)	0(0%)	0(0%)	0(0%)
39	1(2%)	0(0%)	0(0%)	0(0%)
54	2(3%)	1(2%)	0(0%)	19(32%)
75	5(8%)	3(5%)	0(0%)	28(47%)
90	15(25%)	9(15%)	15(25%)	31(52%)
104	25(42%)	30(50%)	27(45%)	40(67%)
	Females*			
14	0(0%)	0(0%)	0(0%)	1(2%)
39	- -	- -	- -	- -
54	1(2%)	1(2%)	0(0%)	8(13%)
75	3(5%)	3(5%)	2(3%)	8(13%)
90	5(8%)	8(13%)	5(8%)	13(22%)
104	12(20%)	20(33%)	13(22%)	21(35%)

[1]Adapted from original report, Vol 1, p. 100 thru 103.
*denominator = 60 animals
-Cumulative mortality not tallied at this time period.

3. Body Weights

Each animal was weighed on the first day of treatment, at weekly intervals for the first 13 weeks and monthly, thereafter.

Body weight gains were depressed in both sexes of the intermediate and high levels when compared to the respective controls. This effect was apparent from the first month of treatment for both sexes of the high level. In the intermediate level, the decreased

4. Water Supply:

Drinking water was supplied to the cages via polyethylene bottles and stainless steel sipper-tubes.

5. Statistics:

The significance differences between treated and control groups were evaluated for body weight, food and water consumption, clinical pathology parameters and organ weights and were assessed by the Student's t-test using pooled within group error variance. Distribution-free tests were applied as appropriate.

Pathological findings were compared using the Fisher's Exact Test.

Possible dosage related effects on survival or tumor incidence were analyzed by pairwise comparison of treated and control groups and by overall trend analysis.

For analysis of tumor incidence, each tumor was classified as fatal (directly or indirectly) or incidental. The effect of the treatment is then tested using the method of Peto et al. Guidelines For Simple, Sensitive, Significant Tests For Carcinogenic Effects in "Long-Term Animal Experiments; in Long-Term and Short-term Screening Assays for Carcinogens: A Critical Appraisal", IARC monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. (Lyon; International Agency for Research on Cancer. Supplement 2; 1980; 311-426.

C. METHODS AND RESULTS:

1. Observations

Rats were inspected at least twice a day (once daily on weekends and public holidays) during treatment. In addition, all animals were handled and superficially palpated once weekly.

Signs

The major treatment related reaction was diarrhea which was recorded in all rats in the high level and in 27/60 males and 45/60 females in the intermediate level. At the high dosage this sign was first observed during week 3 and at the intermediate dosage, sporadically during week 4.

Palpable Swellings

At all dose levels, the incidence of palpable swellings subsequently diagnosed as neoplasms appeared to be similar between treated and control animals.

///

Table 6

Mean Food Consumption(gm/animal/week) at Selected Intervals During the 104 Week Study[1]

	Group and Sex							
Week	1M	2M	3M	4M	1F	2F	3F	4F
7	143	143	148	155[c]	107	111	120[c]	120[c]
25	142	142	147	157[c]	97	99	104[b]	111[c]
54	135	139	149[c]	158[c]	104	105	108	120[c]
74	125	133	135[b]	172[c]	107	106	109	123[c]
102	131	130	134	153[b]	105	105	108	116[a]
Mean^	136	138	140	155	102	104	108	115
%^^	--	6	8	37	--	2	6	15

[1] adapted from original report Vol 1, p. 126 thru 130.
[a] significantly different $p < 0.05$
[b] significantly different $p < 0.01$
[c] significantly different $p < 0.001$
^ Mean of observations throughout. No statistical calculations were performed.
^^ Percent difference compared to control.

5. Food Efficiency

Efficiency of food conversion (ratio between body weight change to the weight of food consumed) was calculated for the first 13 weeks of the study.

An equivocal decrease in the mean food conversion efficiency was noted in the intermediate and high level females when compared to the respective controls.

6. Compound Consumption

Compound consumption expressed as mg/kg/day was calculated for each group/sex (Table 7).

body weight gain, throughout the study, was 11% for males and 22% for females. For the high dose group, the corresponding depression was 22% for males and 34% for females (Table 5).

Table 5

Mean Body Weights (gm) at Selected Time Intervals Throughout the 104 Week Study[1]

	Group and Sex							
Week	1M	2M	3M	4M	1F	2F	3F	4F
0	108	114[a]	106	108	92	97[b]	95	95
4	228	236[b]	224	214[c]	147	151[b]	149	144[a]
7	273	279[a]	265[b]	251[c]	164	170[c]	165	160[a]
29	363	371	349[b]	322[c]	199	203[a]	197	192[c]
54	387	394	370[c]	342[c]	218	221	210[c]	202[c]
74	385	391	368[c]	349[c]	236	241	222[c]	209[c]
104	367	376	336[c]	311[c]	255	259	222[c]	203[c]
Change[d]	259	262	230	203	163	162	127	108
%[e]	--	0%	-11	-22	--	0%	-22	-34

[1]Adapted from original report, Vol 1, p. 118 thru 125.
[a]significantly different from control, $p < 0.05$
[b]significantly different from control, $p < 0.01$
[c]significantly different from control, $p < 0.001$
[d]change in weight (gm) from 0 week value
[e]percentage difference in mean body weight gain compared to control value.

4. Food Consumption

The weight of the food consumed by each cage of rats was recorded weekly for the first 13 weeks of treatment and monthly, thereafter. The mean group intake was calculated at each time period.

Mean food intake was 37% and 15% higher, respectively, in the high level male and female animals throughout 104 weeks when compared to the controls. The increased consumption started about week 7. Mean food intake in the low and intermediate levels were considered to be unremarkable throughout the study (Table 6).

Table 8

Mean Water Consumption (ml/animal/week) at Selected Intervals Throughout the Two Year Study[1]

Week No.	Group and Sex 1M	2M	3M		1F	2F	3F	4F
1	151	144	155	153	119	122	119	133[c]
7	145	148	192[b]	277[c]	126	129	176[b]	202[c]
25	132	136	187[b]	315[c]	110	116	159[c]	241[c]
54	123	126	197[c]	403[c]	108	115	167[c]	320[c]
74	128	133	165[c]	451[c]	126	136	161[c]	331[c]
102	140	158	185[a]	372[c]	145	149	163	276[c]
Mean[d]	137	141	176	342	122	127	160	254
%[e]	---	3	29	149	---	4	31	108

[1]Adapted from original report, Vol 1, p. 134 thru 138
[a]significantly different from control, $p < 0.05$
[b]significantly different from control, $p < 0.01$
[c]significantly different from control, $p < 0.001$
[d]Mean of observations throughout study. No statistical calculations performed.
[e]Percent difference compared to the control.

8. Ophthalmoscopy:

Before the start of the study, the eyes of rats not selected for the clinical pathology examination were examined by means of a Keeler direct ophthalmoscope 20 minutes after the instillation of 0.5% Tropicamide. At 3, 6, 12, 18 and 24 months, the eyes of the Groups 1 and 4 (control and high level, respectively) were similarly examined.

The eyes of both male and female animals were unremarkable throughout the course of the study.

9. Clinical Pathology:

Blood samples from 10 rats of each sex per group were taken for hematology and blood chemistry (excluding hormones) at approximately 3, 6, 12, 18 and 24 months of treatment from all groups. Blood samples from an additional 10 rats per sex per group were taken for the measurement of T3 and T4 at the same time periods. If possible, blood was collected from the same animals at each examination, the animals being selected prior to the commencement of treatment.

Table 7

Mean Compound Consumption for Weeks 1-104[a,b,c]

Group	Males (mg/kg/day)	Females (mg/kg/day)
1	0.0	0.0
2	3.2	3.8
3	27.2	32.9
4	93.1	101.4

[a]Adapted from original report, Vol 1, p. 131 thru 132.
[b]Calculated from the average food consumption and body weight at all time intervals during the study.
[c]Mean compound consumption calculated by the reviewer.

7. Water Consumption:

The amount of water drunk by each cage of rats was recorded weekly for the first 13 weeks of treatment and monthly, thereafter. Group means were calculated at each time period.

Mean water consumption in males was increased by 29% at the intermediate and 149% at the high level. In females, the increase was 31% and 108%, respectively, at the intermediate and high level when compared to the control (Table 8).

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Table 10

Clinical Chemistry Parameters Evaluated During the 104 Week Study

X Blood creatinine
X Blood urea nitrogen*
X Cholesterol*
X Glucose (fasting)*
X Total serum protein*
- Triglycerides*
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Albumin*
X Globulin
X Gamma glutamyl transpeptidase
X Creatine phosphokinase
X Alkaline phosphatase
X Bilirubin (Total)
X Chloride *
X Potassium*
X Sodium*
X Calcium*
X Triiodothyronine (T3)
X Thyroxine (T4)
X Uric Acid
X Phosphorous*

Calcium, phosphorous and cholesterol were measured in millimoles per liter (mM/L). Enzymes were measured in international units per liter (IU/L) while thyroxine (T4) and triiodothyronine (T3) were measured in nanomoles per liter (nM/L). Total protein, albumin and globulin were measured in gram/litter (gm/L). Creatinine was measured in micromoles/liter (uM/L).

The animals were fasted overnight prior to the drawing of blood samples. The blood samples were taken from the retro-orbital sinus with each rat under ether anesthesia. EDTA or citrate (for hematology) or heparin (for blood chemistry) were used as anticoagulants.

a. Hematology:

The parameters marked with an (X) were determined while those marked with a (-) were not evaluated. Parameters marked with an (*) were designated in the latest guidelines (Table 9).

Leukocyte counts (total and differential) were performed in Groups 1 and 4 only.

Table 9

Hematology Parameters Evaluated During the 104 Week Study

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Erythrocyte count (RBC)*
X Leukocyte count (WBC)*
X Leucocyte differential Count*
- Prothrombin Time*
X Platelet Count*

Results

No signs of direct toxicity on the hematological system were present. Changes in the erythrocyte and leukocyte parameters in both males and females were sporadic and inconsistent and did not reflect any changes brought about as a results of treatment with MAA.

b. Clinical Chemistry:

Clinical chemistry parameters determined in the study were designated by an (X) while those marked with a (-) were not evaluated. The parameters marked with an (*) were designated in the latest guidelines (Table 10).

Table 11

Mean Clinical Chemistry Parameters at Selected Time Intervals^

Group /Sex	Plasma Proteins Total gm/L	Alb	Ca mM/L	P	SGOT IU/L	Chol mM/L	Creat uM/L	T3 nM/L	T4
3 Months									
1F	75	37	-	-	29	1.9	-	-	-
2F	75	36[b]	-	-	28	1.9	-	-	-
3F	72[a]	35[b]	-	-	30	1.8[b]	-	-	-
4F	71[b]	35[b]	-	-	24[b]	1.6[b]	-	-	-
6 Months									
1M	64	--	2.5[b]	-	-	-	-	1.9	40
2M	67[a]	--	2.6[b]	-	-	-	-	1.5[a]	34
3M	63	--	2.5	-	-	-	-	1.3[c]	30[b]
4M	62	--	2.4[c]	-	-	-	-	1.4[b]	32[b]
1F	68	36	2.5	-	39	1.8	-	-	31
2F	69	37	2.5	-	32	1.7	-	-	23[a]
3F	65	34	2.4	-	39	1.6[a]	-	-	30
4F	59[a]	33[a]	2.3[c]	-	28[b]	1.5[c]	-	-	32
12 Months									
1M	72	39	-	-	68	2.5	-	-	-
2M	73	38	-	-	94[a]	2.4	-	-	-
3M	71	38	-	-	54	1.9[c]	-	-	-
4M	70[a]	37[a]	-	-	47[b]	2.0[b]	-	-	-
1F	74	42	2.8	-	55	2.8	56	-	19
2F	75	43	2.8	-	47	2.6	60	-	20
3F	72	42	2.8	-	47	2.3[c]	55	-	19
4F	68[c]	37[c]	2.6[c]	-	33[c]	1.9[c]	61[a]	-	37[c]
18 Months									
1M	-	-	-	1.4	-	2.6	53	-	-
2M	-	-	-	1.4	-	2.9	56[a]	-	-
3M	-	-	-	1.6[a]	-	1.8[b]	58[a]	-	-
4M	-	-	-	1.7[a]	-	1.9[b]	63[a]	-	-
1F	77	41	2.8	-	-	2.9	-	-	24
2F	75	41	2.8	-	-	2.7	-	-	25
3F	76	40	2.8	-	-	2.6	-	-	39[b]
4F	68[c]	35[c]	2.6[a]	-	-	2.2[c]	-	-	36[a]

^ Adapted from original report, Vol 1, p. 150 thru 160.
[a] significantly different from control, P < 0.05
[b] significantly different from control, p < 0.01
[c] significantly different from control, p < 0.001

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Evidence of the poor condition of the high dose female group was indicated by decreased concentration of total protein and albumin in the plasma. This was accompanied by a reduction in plasma cholesterol level in this group compared to the controls. These effects were noted in all but the 24 month examination (Table 11).

When compared to the corresponding controls, the intermediate level females showed decreased total protein and albumin at 3 months and decreased cholesterol at 6 and 12 months. The intermediate level males showed decreased cholesterol at 12 and 18 months compared to the control (Table 11).

Calcium levels showed a marginal but statistically significant decrease in the high level females at 6, 12 and 18 months (Table 11).

Thyroxine (T4) showed decreases in the intermediate and high level males at 6 months. The intermediate dose females showed decreases at 18 months while the high dose females showed decreases at 12 and 18 months (Table 11). At 24 months, this parameters was considered to be unremarkable. The toxicological significance of these changes were unknown.

At 24 months, gamma glutamyl transpeptidase (GGTP) in the high level male was decreased from 14.40 IU/L in the control to 2.64 IU/L. The toxicological significance of this change was unknown.

Although statistical significance was noted in other parameters, they were considered to be of little toxicological significance due to the equivocal nature of the response and/or the lack of a time or dose relationship, e.g. SGPT, and triiodothyronine (T3) showed equivocal decreases, while creatinine and phosphorous showed equivocal increases at various time periods (Table 11).

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Table 13

Mean Urinalysis Parameters[1]

Group and Sex

Group	1M	2M	3M	4M	1F	2F	3F	4F
3 Months								
Volume[2]	3.5	3.7	3.2	1.8[c]	2.7	2.7	2.7	1.2[c]
pH	7.3	7.1	7.0	6.4[c]	--	--	--	--
Sp. Grav.	1.060	1.056	1.063	1.079[c]	1.052	1.054	1.060	1.071[b]
6 Months								
Volume	3.4	3.3	2.8[a]	2.1[c]	--	--	--	--
pH	7.0	6.9	6.5[c]	6.2[c]	--	--	--	--
Sp. Grav.	1.061	1.062	1.075[c]	1.074[c]	--	--	--	--
12 Months								
Volume	3.5	2.8	3.2	2.1[a]	3.0	3.2	2.0[c]	2.2
pH	6.9	7.0	6.8	6.2[c]	--	--	--	--
Sp. Grav.	--	--	--	--	1.051	1.053	1.069[c]	1.053
18 Months								
Volume	4.1	4.0	4.0	3.6[a]	3.6	3.5	2.5[b]	1.6[c]
pH	7.1	7.0	7.0	6.0[c]	--	--	--	--
Sp. Grav.	1.053	1.056	1.053	1.060[b]	1.047	1.047	1.057[c]	1.052
24 Months								
Volume	--	--	--	--	4.5	5.2	4.3	2.5[b]
pH	6.9	6.7	6.8	6.3[b]	6.2	6.1	6.1	5.6[c]
Sp. Grav.	--	--	--	--	1.037	1.036	1.043	1.055[c]

[1]Adapted from original report, Vol 2, p. 161 thru 170.
[2]Volume in ml
[a]significantly different from control $p < 0.05$
[b]significantly different from control $p < 0.01$
[c]significantly different from control $p < 0.001$

10. Sacrifice and Pathology:

Animals in extremis and those that completed their scheduled test period were sacrificed by carbon dioxide inhalation.

All animals that died or were scheduled for sacrifice were subject to gross and pathological examination. The checked (X) tissue were collected for gross and histological examinations in the control

c. Urinalysis:

Urine samples were collected after approximately 3, 6, 12, 18 and 24 months of treatment from those rats selected for withdrawal of blood samples.

The rats were deprived of drinking water for 3.5 hours on the day of collection and were placed individually into metabolism cages with out food and water. Urine was collected for 16.5 hours.

The following parameters marked with an (X) were examined while those marked with a (-) were not. Parameters marked with an (*) were required by the guidelines (Table 12).

Table 12

Urine Parameters Evaluated During the 104 Week Study

X Appearance*	X glucose*
X Volume*	X ketones*
X Specific Gravity	- bilirubin
X pH	X blood (occult)*
X Sediment (microscopic)*	- nitrate
X Protein*	X urobilinogen
X Sediment*	

Urine sediment was examined microscopically for:

epithelial cells
polymorphonuclear leukocytes
red blood cells
casts
crystals
other abnormal components.

Results

The high level males voided a smaller volume of urine at 3, 6, 12 and 18 months. Specific gravity was increased at all time periods except the 12 and 24 month examinations. The urine of this group was found to be more acidic at all time intervals (Table 13).

In the female high dose group, a decreased volume of urine was noted at 3, 18 and 24 months with an increase specific gravity at 3 and 24 months; pH was decreased at 24 months (Table 13).

The intermediate dose level males showed decreased volume, pH and specific gravity at the 6 month examination only. The intermediate dose level females showed increased specific gravity at 12 and 18 months (Table 13).

The liver was lighter in weight in the male high dose group. Covariant analysis indicated this effect was caused by reduced body weight. In females, the liver/body weight ratio was increased at the intermediate and high dose levels when compared to the respective control and appeared due to a decreased total body weight (Table 16). Liver pathology was not remarkable compared to the respective control.

The kidneys were significantly lighter in weight in males of the high and intermediate levels. The kidney/body weight ratio was increased in the intermediate and high dose level female animals versus the control (Table 15, 16).

The heart was significantly heavier in females of the intermediate and high dosage groups. The heart/body weight ratio was increased in the high dose males and the intermediate and high dose females (Table 16). No histopathological evidence of treatment related cardiac toxicity was found (Table 15, 16).

In the high level females, the adrenal/body weight ratio was increased versus the control (Table 16).

Table 15

Mean Organ Weight at Necropsy^

Group & Sex	Brain (gm)	Liver (gm)	Kidney (gm)	Heart (gm)	Thyroid (gm)
1M	2.0	14.3	3.2	1.2	26
2M	2.0	15.4	3.2	1.1	27
3M	1.9[c]	14.3	3.0[b]	1.1	22[a]
4M	1.9[c]	13.0[a]	2.8[c]	1.2	23
1F	1.8	9.7	2.2	0.84	24
2F	1.8	10.0	2.3	0.84	24
3F	1.7[c]	9.6	2.3[b]	0.90[a]	19[c]
4F	1.6[c]	9.5	2.1	0.90[b]	18[c]

^ Adapted from original report Vol 1, p. 177 thru 178.
[a] significantly different from control, $p < 0.05$
[b] significantly different from control, $p < 0.01$
[c] significantly different from control, $p < 0.001$

and high levels. Tissues designated by (^^) and the target organs (cecum, colon and rectum) from the low and intermediate levels were also microscopically examined. The (XX) organs were weighed. Organs and tissues marked with a (*) were required by the guidelines (Table 14).

Table 14

Organs and Tissues Examined Histopathologically at the Terminal Sacrifce

Digestive	Cardiovas./ Hematology	Neurologic
X tongue	X aorta*	XX brain*
X esophagus*	XX heart*	- peripheral nerve*
X stomach*	X bone marrow*	X spinal cord (3 levels)
X duodenum*	X lymph nodes*	X sciatic nerve
X jejunum*	cervical/	X pituitary*
	mesenteric	X eyes* & optic nerve*
X ileum*	X spleen*	Glandular
X cecum*^^	X thymus*	XX adrenals*
X colon*^^	Urogenital	X parathyroids*^^
X rectum*^^	XX kidney*^^	XX thyroids*^^
XX liver*	X urinary bladder	Other
X pancreas*	XX testes(b)*	X bone*
Respiratory	X prostate*	X skeletal muscle*
X trachea*	X seminal ves.	X skin*
X lung*^^	X ovaries(a)*	X unusual lesions*
	X uterus*	- Harderian gland
		X salivary gland*

(a) with fallopian tubes
(b) with epididymis
X examined microscopically
XX weighed and examined microscopically
^^ microscopic examination from low and intermediated level
* specified by the guidelines

a. Organ Weights:

The brain was decreased in weight in both treated sexes of the intermediate and the control groups. The brain/body weight ratio was significantly heavier in the intermediate and high level females and the high dosage males. No pathological evidence was noted to account for these differences (Table 15, 16).

The thyroid weighed significantly less in females of the intermediate and high levels when compared to the respective control. No pathological evidence was noted to account for this weight difference (Table 15).

Other MAA related pathology secondary to the intestinal lesions included inflammation, congestion and enlargement of the small lymph-nodes and reduced size and capsular thickening of the spleen.

c. Histopathological Findings:

1. Non-neoplastic lesions

Histopathological detectable toxic effects due to MAA treatment were noted mostly in the high dose groups from week 1 - 59 and in the intermediate dose level group at 60 - 104 week of treatment. The observed pathological changes indicated that the large intestine (cecum, colon, rectum) was the principal target organ of the direct irritant (toxic) effect of MAA which caused large intestinal perforating ulcers and leakage of the intestinal contents into the abdominal cavity with secondary irritation. The cecum and rectum were more affected than the colon. Other organs (stomach, ileum and jejunum) manifested signs of direct irritation. Other gastro-intestional segments and different abdominal organs were involved in the reaction to MAA treatment but most likely the lesions were due to a secondary irritation of the adjacent organs from the leaking intestional contents; the following organs were affected: duodenum, ureter, testes, epididymis, seminal vesicles, prostate, uterus, urinary bladder, peritoneum and pancreas.

The primary organs and tissues affected by MAA were listed below.

Abdominal Wall and Cavity

Week 1 - 59

Acute or subchronic peritonitis and atrophy of fat pads.

Week 60 - 104

High dose group - acute or chronic peritonitis
Intermediate and high dose group - Atrophy of abdominal fat pads

Colon, Caecum, Rectum

Week 1 - 59

Acute inflammation, mucosal vascular congestion, cuboidal to squamous metaplasia of the epithelial columnar absorptive cells, mucosal ulceration which sometimes became perforated with regenerative hyperplasia, increased presence of goblet cells in the intestinal gland and occasional focal extensions of glands (crypts) within the wall (diverticula).

Weeks 60 -104

The same range of pathological observations as in rats during weeks 1-59. Frequency of lesions was dose related and limited mainly to the high dose groups.

Table 16

Mean Organ/Body Weight Ratio at Necropsy^

Group & Sex	Brain (%)	Liver (%)	Kidney (%)	Heart (%)	Adrenal (% x 1000)
1M	0.55	4.0	0.87	0.32	15.6
2M	0.54	4.2	0.86	0.31	15.1
3M	0.57	4.4	0.90	0.34	15.7
4M	0.61[c]	4.2	0.92	0.36[a]	18.7
1F	0.72	3.8	0.86	0.33	22.5
2F	0.71	3.9	0.88	0.35	23.6
3F	0.76[a]	4.3[b]	1.03[c]	0.40[c]	23.7
4F	0.81[c]	4.7[c]	1.04[c]	0.45[c]	29.2[c]

^adapted from original report Vol 1, p. 177 thru 178.
[a]significantly different from the control. $p < 0.05$
[b]significantly different from the control, $p < 0.01$
[c]significantly different from the control, $p < 0.001$

b. Gross Pathological Observations

Animals dying or sacrificed during the study evidenced emaciation and dehydration associated with reduced fat pads in the abdominal cavity. In the stomach, small intestine (duodenum, jejunum, ileum) and large intestine (cecum, colon, rectum), the wall was thickened, while the mucosa was edematous, congested, hemorrhagic, necrotic, ulcerated or perforated; the serosa was congested while the lumen was distended and contained foamy, mucoid or hemorrhagic contents. Frequently the intestinal ansae adhered to each other or to adjacent abdominal organs.

As a sequela of MAA-induced intestinal perforating ulcers, lesions in adjacent organs were observed. These included: induration, nodules or edema of the prostate; small, soft, firm and bluish testes; hydronephrosis of the kidney; discoloration, adhesions, distension, hemorrhagic contents and congested mucosa of the urinary bladder; epididymal abscess; variation in the size of seminal vesicles; distension and thickened wall of the ureter.

Week 1 - 59

In both sexes of the high dose level - various grades of basophilic cells lining the tubules. Hydronephrosis, cortical tubular cystic dilatation, pyelonephritis and papillary necrosis.

Week 60 - 104

Same as above, with increased severity of progressive glomerulonephropathy in the high and intermediate dose level.

2. Neoplasms

Neoplasms that showed a dose response and were greater than the NTP historical control values were noted in Table 17.

Brain and Spinal Cord: Astrocytomas were observed in the intermediate (1/60) and high (1/60) level female animals versus 0/60 in the control. The NTP background data indicated a 0.5% incidence of this type of tumor. These tumors were judged to be sporadic and not related to treatment (Table 17).

Cecum and Rectum: Mesenchymal tumors - leiomyomas were observed sporadically in the cecum of high level females (1/58) versus 0/59 in the control. Leiomyosarcomas were noted in the high level males (1/60) and females (1/60) compared to 0/60 in the respective controls. Leiomyomatous tumors were not mentioned as occurring spontaneously in the NTP background data. Due to the sporadic nature of this finding, these tumors were judged as not being related to treatment (Table 17).

Parathyroid: Increased incidence of adenomas were observed in the intermediate (4/53) and high (4/45) level males versus 1/52 in the male controls. High level females had an incidence of 4/45 versus 0/46 in the controls. The NTP background data showing a 0% incidence for Fischer F344 rats emphasizes the rarity of this tumor (Table 17).

The parathyroid adenomas showed a significant positive trend in males ($p < 0.01$) and in the data combined by sex ($p < 0.001$). Upon applying the Fisher exact test, the results associated with the adenomas of the parathyroid gland were not significant with regard to each sex, but the results combined for the sexes were significant ($p < 0.01$). Each of the significant results remained significant at the 5% level even when Bonferroni correction was applied.

Thyroids

Week 1-59

Increased height of the lining of the follicular epithelium as compared to controls was observed in both sexes of the high dosage group.

Week 60 - 104

Same as above but also noted in the intermediate dose group.

Spleen

Week 1 - 59

Slight to moderate degree of depletion of lymphocytes from the white pulp was observed.

Week 60 - 104

Same as above but not in the intermediate dose.

Thymus

Week 1 - 59

Relatively earlier appearance of the normally age-associated atrophy.

Week 60 - 104

Same as above

Bone Marrow

Week 1 - 59

Reduced cellularity

Week 60 - 104

As above, but observed in the intermediate dose level as well.

This finding was found in males with a trend in females.

Kidneys

Severe kidney pathology was noted as a sequela to the ureters being attacked by the leaking intestinal contents and inflammation developing which partially occluded the urinary tract.

The percent mortality in males was increased throughout the study at the high versus the control level. In the females, although mortality was similar at the intermediate (22%) and control (20%) levels, an increase was observed at the low level (33%). High level animals had a mortality of 35%. This sporadic increase in mortality at the low level without other symptomatology or pathological evidence of toxicity was not considered to be of any toxicological significance (Table 4).

The primary target organ for MAA induced toxicity was the large intestine. Functional impairment of this organ was manifested by a decreased food consumption in the high level males of 37% and in the high level females by 15% when compared to the corresponding controls (Table 6). Males had reduced body weight gains of 11% and 22% at the intermediate and high levels, respectively, when compared to the control. Females had reduced body weight gains of 22% and 34% at the intermediate and high levels, respectively, when compared to the respective controls (Table 5).

Compared to controls, water intake was markedly elevated in the high level by 149% in males and 108% in females throughout the study. In the intermediate dose level a 29% increase was seen in the males and a 31% increase was seen in the females versus the controls (Table 8). The excess imbibed water was eliminated through the gastro-intestinal tract.

Urine volume was reduced and the specific gravity was elevated in the high level males at 3, 6, and 18 months when compared to the respective controls. At 12 months, the volume was decreased, but no change was noted in the specific gravity. In the high level males, pH was decreased at all time intervals. In the high females, urine volume was reduced at 3, 18, and 24 months, specific gravity elevated at 3 and 24 months and urine pH decreased at 24 months. In the intermediate females, urine volume was decreased at 12 and 18 months (Table 13).

As noted below, clinical chemistry parameters of the female showed depressed total protein and albumin values at the high level. This effect might have been a reflection of the debilitated condition of these animals. Cholesterol was depressed at all but the 24 months examination (Table 11).

3 month - depressed total protein (F), albumin (F), cholesterol (F)
6 month - depressed total protein (F), albumin (F) cholesterol (F), T4 (M)
12 month - depressed total protein (M,F), albumin (M,F) cholesterol (M,F), T4 (F)
18 month - depressed total protein (F), albumin (F), cholesterol (M,F), T4 (F)

At the intermediate dose, males showed depression of cholesterol at 12 and 18 months and females at 6 and 12 months. Total protein and albumin were depressed in the intermediate level males only at 12 months (Table 11).

Table 17

Incidence of Neoplasms in the Brain and Spinal Cord, Large Intestine and Parathyroids[a,b]

Group and Sex							
1M	2M	3M	4M	1F	2F	3F	4F
Brain and Spinal Cord - Astrocytoma							
-	-	-	-	0/60[1]	0/60	1/60	1/60
Caecum - Leiomyoma							
-	-	-	-	0/59[2]	0/59	0/59	1/58
Rectum - leiomyosarcoma							
0/58[3]	0/57	0/60	1/60	0/60[4]	0/59	0/59	1/60
Parathyroid - adenoma							
1/52[5]	0/49	4/53	4/45	0/46[6]	0/44	0/40	4/45

[a]Incidence of neoplasms having increased incidence in the present study and outside the range of incidences in LSRI and NTP background data.
[b]The denominators represent the actual number of tissues/organs examined.

[1]NTP background rate - females 0.5%
[2]NTP background rate - females 0%
[3]NTP background rate - males 0%
[4]NTP background rate - females 0%
[5]NTP background rate - males 0.1%
[6]NTP background rate - females 0.1%

D. DISCUSSION:

Methanearsonic acid (MAA) was incorporated into the diet of 4 groups of 60 Fischer F344 rats per sex at concentrations, respectively, of 0 (Control), 50, 400 or 1300 ppm for 104 weeks. The 1300 ppm concentration was reduced to 1000 ppm during week 53 and to 800 ppm at week 60 because of excessive mortality. Following the second reduction in concentration, a decrease in mortality was observed.

Possible treatment related effects on organ weights at terminal sacrifice were a reduction in thyroid weight in females in the high and intermediate levels. Increased height of the lining of the follicular epithelium as compared to controls was observed in both sexes of the intermediate and high levels (Table 15).

Neoplastic Lesions:

Parathyroid: In male animals, an increased incidence of adenomas were observed in the intermediate (4/53) and high (4/45) dose groups. One (1) of 52 male control rats also evidenced a parathyroid adenoma. Parathyroid adenomas were also observed in 4/45 high level females versus 0/46 in the control group (Table 17).

Calcium levels of the high level females showed a marginal, but statistically significant depression at 6, 12 and 18 months which might have been related to the above finding (Table 11).

The adenomas of this gland showed a significant positive trend in males ($p < 0.01$) and in the data combined by sex ($p < 0.001$). Upon applying the Fisher exact test, the results associated with the adenomas of the parathyroid gland were not significant with regard to each sex, but the results combined for the 2 sexes were significant ($p < 0.01$). Each of the significant results remains significant at the 5% level even when Bonferroni correction was applied.

A suggestion of a carcinogenic response for parathyroid adenomas in animals of both sexes is, therefore, warranted.

E. CONCLUSIONS:

Methanearsonic acid (MAA) was incorporated into the diet of 4 groups of 60 Fischer F344 rats per sex at concentrations of 0 (Control), 50, 400 or 1300 ppm for 104 weeks. The 1300 ppm concentration was reduced to 1000 ppm during week 53 and to 800 ppm at week 60 because of excessive mortality.

Mortality was increased in the high level animals when compared to the respective controls.

Reduced body weight gain occurred in males at the high level and in females at both the intermediate and high levels.

High level animals had acute inflammation, mucosal ulceration and perforations of the large intestine (cecum, colon and rectum). The abdominal wall showed evidence of acute or chronic peritonitis. The intermediate dose was similarly but sporadically affected.

A reduction in the thyroid weight of the intermediate and high level females and the intermediate level males was observed together with an increase height of the thyroid follicular epithelium of the high and intermediate levels of both sexes.

Calcium levels of the high level females showed a marginal, but statistically significant depression at 6, 12 and 18 months. This effect might have been related to the apparent increases in thyroid adenomas seen in the high level females but does not provide any insight into the observed adenomas which occurred in the intermediate and high level males (Table 11).

Other clinical chemistry and urine parameters showed variations throughout the study but were not considered to be of any toxicological significance either because of the equivocal nature of the changes or the lack of a dose or time relationship.

Gross necropsy revealed lesions mainly in the high level animals which consisted of emaciation and dehydration associated with reduced fat pads in the abdominal cavity. In the stomach, small intestine (duodenum, jejunum, ileum) and large intestine (cecum, colon, rectum), the intestinal wall was thickened, while the mucosa was edematous, congested, hemorrhagic, necrotic and ulcerated. The lumen was distended and contained foamy, mucoid or hemorrhagic contents. Frequently the intestinal ansae adhered to each other or to adjacent abdominal organs.

As a sequela of MAA induced intestional perforating ulcers, lesions were observed in adjacent organs, such as the prostrate, testes, kidneys, urinary bladder, epididymis, seminal vesicles and ureter.

Histopathology were noted in the high levels and sporadically in the intermediate levels. The observed pathological changes indicated that the large intestine (caecum, colon, rectum) was the principal target of the direct irritant (toxic) effect of MAA. The cecum and rectum were more severely affected than the colon. These organs showed acute inflammation, mucosal congestion, inflammation and ulceration which sometimes became perforated. The abdominal wall showed acute or subchronic peritonitis and serous atrophy of the fat pads.

Other organs (stomach, ileum and jejunum) also manifested signs of direct irritation. Other gastro-intestional segments and different abdominal organs were involved in the reaction to MAA treatment but most likely the lesions were due to a secondary complications e.g. due to large intestinal perforating ulcers and leakage of the intestinal contents into the abdominal cavity with secondary irritation of the adjacent organs such as the duodenum, ureter, testes, epididymis, seminal vesicles, prostate, uterus, urinary bladder, peritoneum and pancreas.

Severe kidney pathology occurred as a sequela to the ureters being attacked by the leaking intestinal contents; inflammation developed which partially occluded the urinary tract. The kidneys were significantly lighter in weight in males of the high and intermediate levels. The kidney/body weight ratio was increased in the intermediate and high dose level female animals versus the control (Table 15, 16).

Page_____ is not included in this copy.

Pages 134 through 141 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

The maximum tolerated dose (MTD) based on a decreased body weight gain for chronic dietary administration of MAA was 400 ppm.

A suggestion of an increased incident of parathyroid gland adenomas was seen in males at the intermediate and high levels and in the females at the high level.

The NOEL = 50 ppm (low level); the LOEL (systemic toxicity) = 400 ppm (intermediate level).

Supplement to Tox Document 009523, review for Accession 41872701, 21-day dermal toxicity study-rabbit. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 9/12/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 9/12/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-Day Dermal Toxicity - Rabbit

DP BARCODE: D265953 SUBMISSION CODE: S579557
PC CODE: 013803 TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (99.4% a.i.)

SYNONYM: Monosodium acid methanearsonate, MSMA

CITATION: Margitich, D., L. Ackerman (1991) Methanearsonic acid - 21-day dermal toxicity study in rabbits. Pharmakon Research International, Inc., Waverly, PA. Laboratory study number PH 430-LI-009-90, March 13, 1991. MRID 41872701. Unpublished.

Margitich, D., L. Ackerman (1991) Methanearsonic acid - 21-day dermal toxicity study in rabbits. Pharmakon Research International, Inc., Waverly, PA. Laboratory study number PH 430-LI-009-90, March 13, 1991. MRID 42659701. Unpublished.

SPONSOR: MAA (MSMA/DSMA) Research Task Force Three, Luxembourg Industries (Pamol), Ltd., Tel Aviv, Israel.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study (MRID 41872701/42659701), methanearsonic acid (99.4% a.i., Batch #0030401) was administered dermally to 5 New Zealand white rabbits/sex/group at doses of 0, 100, 300, or 1000 mg/kg/day for 6 hours/day, 5 days/week for 21 days.

There were no treatment related effects on mortality, clinical signs, mean body weight, mean body weight gain, hematology, urinalysis, gross necropsy findings, or histopathology findings. Opthalmological examinations were not conducted. Food consumption was statistically decreased during one interval at 100 mg/kg/day and during two intervals at 1000 mg/kg/day (none of the intervals were not defined). Mean cholesterol concentration was statistically ($p<0.05$) decreased in males at 300 and 1000 mg/kg/day. In females, mean cholesterol concentration was decreased at 100 and 300 mg/kg/day as compared to controls, and increased at 1000 mg/kg/day, but statistical significance was not attained.

EXECUTIVE SUMMARY

014343

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MONOSODIUM METHANEARSONIC ACID - (MSMA)
(METHANEARSONIC ACID)

STUDY TYPE: 21-DAY DERMAL TOXICITY – RABBIT
[OPPTS 870.3200 (§82-2)]
MRID NO. 41872701

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: H T Borges
Date: MAY 22 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle. LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

1/2

CASWELL FILE

009523

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 5-18-92
Section IV, Tox. Branch II (H7509C)

Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A. Doyle 5/15/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

Study Type: 21-Day Dermal Toxicity Study - Rabbits (§82-2)

Test Material: Methanearsonic Acid

Synonyms: monosodium acid methanearsonate

Tox. Chemical No.: 582 **MRID No.:** 418727-01
Identification No.: 013803-042519 **HED Project No.:** 1-1901

Sponsor: MAA (MSMA/DSMA) Research Task Force Three
Luxembourg Industries (Pamol), Ltd.
Tel Aviv, Israel

Testing Facility: Pharmakon Research International, Inc.
Waverly, PA

Title of Report: Methanearsonic Acid - 21-Day Dermal Toxicity Study in Rabbits

Study Number: PH 430-LI-001-90

Authors: Dennis J. Margitich and Larry J. Ackerman

Report Issued: March 13, 1991

Conclusions:

Methanearsonic acid was placed on the intact skin of the back (hair clipped) of rabbits at doses of 0 (deionized water control), 100, 300 and 1,000 mg/kg for 6 hours (occlusive dressing) 5 days/ week for 3 weeks. There did not appear to be any skin changes or other definitive effects caused by test article administration.

Systemic Toxicity No Observed Effect Level (NOEL) = 1,000 mg/kg (Limit Dose)
Systemic Toxicity Lowest Observed Effect Level (LOEL) = not attained (>1,000 mg/kg, HDT)

Dermal Toxicity No Observed Effect Level (NOEL) = 1,000 mg/kg
Dermal Toxicity Lowest Observed Effect Level (LOEL) = not attained (>1,000 mg/kg, HDT)

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In the results section of the original review, it is mentioned that the kidney to body weight ratio and liver to body weight ratio were significantly ($p<0.05$ or $p<0.01$) increased in females at the 100 mg/kg/day dose level, and the liver to body weight ratio was significantly ($p<0.05$ or $p<0.01$) increased in females at the 1000 mg/kg/day dose level. It is important to note that the absolute liver and kidney weights were similar to control among all dose groups. These organ weight findings are considered incidental. Body weights of female rabbits in the 1000 mg/kg/day group at initiation of the study were slightly lower than control (2249 g and 2180 g for control and 1000 mg/kg/day, respectively). Although body weight gain was similar among all groups, body weights of the high dose group continued to be slightly lower for the duration of the study (at termination 2697 g vs 2509 g for control and 1000 mg/kg/day, respectively).

The systemic toxicity LOAEL > 1000 mg/kg/day. The systemic toxicity NOAEL was ≥ 1000 mg/kg/day.

There was no edema or erythema noted at the exposure sites of any dose group. There were no histological dermatopathology findings at the 1000 mg/kg/day dose level as compared to the control group. **The dermal irritation LOAEL > 1000 mg/kg/day. The dermal irritation NOAEL ≥ 1000 mg/kg/day.**

This study was previously classified unacceptable but has been upgraded to acceptable based on submitted analytical data indicating that the purity MSMA in the aqueous solution used for dosing was > 99%.

009523

Classification: **Core Supplementary** - The Registrant needs to provide replacement pages and analytical data acceptable to the Agency in order to upgrade this Report to **Core Minimum.**

This study does not satisfy the Guideline Requirements (§82-2) for a 21-day dermal toxicity study in rabbits.

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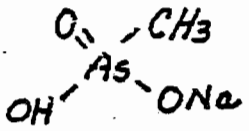
I. MATERIALS AND METHODS

A. Test Article

Name: Methanearsonic acid, monosodium acid methanearsonate

Formula:



Lot Number: 0030401 (Batch Number)
Purity: 99.44%
Appearance: white crystalline flake

Vehicle: deionized water (control group only - 1 ml)

B. Animals

Albino New Zealand White Rabbits from Hare-Marland, Hewitt, NJ were used in this study. There was a 10 day period of acclimation. The animals weighed 2.05-2.56 kg at study initiation and were assigned to treatment groups according to body weights by a table of random numbers. Rabbits were individually housed. "Every attempt was made to maintain a temperature of 20°C ± 3°C (63°F to 74°F) and a humidity of 30 to 70%." "Temperature and humidity readings were within range throughout the study." There was a 12 hour light/dark cycle. Food and water were available ad libitum.

C. Dosing

Four groups of animals (5/sex/group) received 0 (vehicle control, deionized water), 100, 300 and 1,000 (Limit dose) mg/kg of the test article. Dosing was 5 days/week for 3 weeks.

At least 10% of the dorsal surface of collared rabbits had hair removed by clipping (repeated as necessary). The test article was applied to the intact skin as received from the Sponsor (white crystalline flake). Gauze patches, moistened with 1 ml of deionized water were then applied to the site. A rubber dam was placed over the patches and the animal was wrapped with an elastic bandage held in place with tape. After 6 hours of exposure, the wrappings, dam and gauze were removed and the test site wiped to remove test article.

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D. Observations

1. Skin - Sites were observed daily prior to dosing as as well as at terminal sacrifice for signs of erythema and edema (scored by the Draize Method - page 90 of the Report).

2. Clinical Signs - Observations were made at least once daily for toxicologic/pharmacologic signs. Mortality checks were made twice daily.

3. Body Weights - These were recorded initially, weekly and at terminal necropsy. Dosing (mg/kg) was based on the most recent body weight.

4. Food Consumption - This was measured on days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

5. Clinical Laboratory - Blood samples for hematology and chemistry were obtained from the central ear artery (pretest) or from cardiac puncture (at sacrifice) after an overnight fast. Urine was collected prior to study initiation and at termination. [NOTE: No collection details were provided in the report.]

   a. Hematology - The following parameters were examined:

Hemoglobin	Platelet Count
Hematocrit	Mean Corpuscular Volume
Erythrocyte Count	Mean Corpuscular Hemoglobin
Total Leucocyte Count	Mean Corpuscular Hemoglobin Concentration
Differential Leucocyte count	

   b. Chemistry - The following parameters were examined:

A/G Ratio	Serum Aspartate Aminotransferase
Calcium	Urea Nitrogen
Phosphorus	Albumin
Chloride	Blood Creatinine
Sodium	Total Bilirubin
Potassium	Total Serum Protein
Fasting Glucose	Total Cholesterol
Serum Alanine Aminotransferase	

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c. Urine - The following parameters were examined:

Occult Blood	Total Bilirubin
Protein	Urobilirubin
Ketone Bodies	Sediment
Appearance	Specific Gravity
Glucose	pH

6. Gross Pathology - All surviving animals were euthanitized by intravenous sodium pentobarbital. A necropsy was performed on all rabbits. The following organs were weighed (weights expressed as absolute, relative to body weight and relative to brain weight): gonads, adrenals, brain, kidneys and liver.

7. Microscopic Pathology - Organs/tissues from the terminal sacrifice were preserved in 10% neutral buffered formalin for "possible future histopathological examination." Evaluation was performed on treated and untreated skin as well as on organs/tissues from all control and high-dose terminally sacrificed animals. The following tissues were preserved:

Gross Lesions	Pancreas	Ileum
Brain (medulla/pons)	Testes	Cecum
cerebellar & cerebral cortex	Ovaries	Colon
	Uterus	Rectum
Pituitary	Epididymides	Gallbladder
Thyroid/parathyroid	Prostate	Urinary Bladder
Thymus	Seminal Vesicles	Cervical Lymph Node
Lungs	Aorta	Mammary Gland
Trachea	Skin (treated & untreated)	Thigh Muscle
Heart		Peripheral Nerve
Sternum (marrow)	Tongue	Eyes
Salivary Glands	Esophagus	Femur
Liver	Nasal Turbinates	Spinal Cord (3 levels)
Spleen	Stomach	Lachrymal Glands
Kidneys	Duodenum	
Adrenals	Jejunum	

II. RESULTS

A. Test Article Purity and Stability

N O T E: Page 13 of the Report states: "The purity, identity, strength and stability of the test article were the responsibility of the Sponsor. Samples of the test article were not required to be submitted to the sponsor for stability analysis, as analysis was previously performed."

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Page 547 of the Report is a "Certificate of Analysis" from Luxembourg Industries (PAMOL) Ltd. This certifies that Methanearsonic Acid - Tech (MAA), Batch 0030401 sent to Pharmakon Research International, Inc., "... has gone through quality control procedures, and was found to meet all specificat[illegible]." The assay % w/w stated to be "99.44%".

R E V I E W E R' S C O M M E N T: No analytical data regarding purity or stability were presented in this report. Therefore, the study is considered to be **Core Supplementary.** If the Registrant provides these analytical data and they are acceptable to the Agency, the Study will be upgraded to **Core Minimum.**

B. Mortality and Clinical Observations

1. Mortality - All rabbits survived until terminal sacrifice after 21 days.

2. Toxicologic/Pharmacologic Signs - There were none which were attributed to test article administration.

3. Skin - No erythema or edema was reported for any animal at any observation time.

C. Body Weights

Body weight and weight gain data are summarized in Table 1.

There were no statistically significant ($p<0.05$ or $p<0.01$) differences in body weights or weight gains between any treated group and the respective control for males or females at any interval. The variations in weight gains and the standard deviation ranges shown in Table 1 indicate the amount of variation of these values within a group as well as from group-to-group. It is not considered that there was any biological difference in body weights or weight gains that were caused by test article administration.

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Table 1

A SUMMARY OF GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 21-DAY DERMAL RABBIT STUDY WITH METHANEARSONIC ACID

	Males				Females			
Day mg/kg =	0	100	300	1000	0	100	300	1000
BODY WEIGHTS (kg)								
0	2.4	2.4	2.4	2.3	2.2	2.2	2.2	2.2
7	2.6	2.5	2.5	2.4	2.4	2.3	2.4	2.3
14	2.7	2.7	2.6	2.6	2.6	2.5	2.6	2.4
20	2.7	2.7	2.8	2.7	2.7	2.6	2.7	2.5
BODY WEIGHT GAIN (g)								
0-7	142	102	103	133	165	13	113	101
7-14	100	105	164	144	221	198	203	100
14-20	54	64	122	81	62	129	119	128
0-20	296	271	389	358	448	340	435	329

NOTE: Standard Deviation ranges (g) for body weights - males = 80-253; females = 93-289

Data extracted or calculated from Report Tables 1 and 2, pages 24-27.

D. Food Consumption

In males, all group mean values at all intervals were similar.

For females, one interval at 100 mg/kg and two intervals at 1,000 mg/kg were statistically ($p<0.05$ or $p<0.01$ lower than the control value. The data were presented as daily mean food consumption, and in almost all instances, the number of g of food consumed for the 3 dosed groups was numerically below the control figures. The test article appeared to have little or no effect on food consumption.

E. Hematology

The only statistically significant differences were in males and were reported to be as follows: mean corpuscular hemoglobin concentration lower at 300 mg/kg, monocytes lower at 300 and 1,000 mg/kg. None of these were considered to be test article related or of biological significance.

There were no differences noted in any other parameters in males.

009523

Group mean hemoglobin and hematocrit values suggested slight anemia in females at 1,000 mg/kg (See Table 2). However, a review of individual animal data indicated that this observation was primarily due to one rabbit, No. 5036 (Table 3).

Table 2

SELECTED HEMATOLOGIC PARAMETERS IN FEMALE RABBITS ADMINISTERED METHANEARSONIC ACID DERMALLY FOR 21-DAYS

Dose mg/kg	Baseline			Terminal		
	Erythroc	Hematocr	Hemoglob	Erythroc	Hematocr	Hemoglob
0	6.5±0.4	43±2.4	13.9±0.5	5.8±0.6	39±2.9	12.4±1.0
100	5.6±0.6	38±3.9	12.5±1.0	5.2±0.5	35±2.5	11.2±0.6
300	5.9±0.3	39±1.1	12.8±0.3	5.4±0.7	37±3.8	11.6±1.3
1,000	6.3±0.5	41±3.5	13.1±1.0	5.1±0.8	33±4.3	10.7±1.1

Erythroc = Erythrocytes ($10^6/mm^3$)
Hematocr = Hematocrit (%)
Hemoglob = Hemoglobin (g/dl)
NOTE: Values are group means ± standard deviations for 5 females
Data extracted from Report Table 10, pages 72 and 73.

Table 3

INDIVIDUAL ANIMAL VALUES FOR ERYTHROCYTES, HEMATOCRIT AND HEMOGLOBIN PARAMETERS IN RABBITS ADMINISTERED METHANEARSONIC ACID AT 1,000 MG/KG FOR 21 DAYS

Parameter	No. =	Baseline					Terminal				
		5036	5037	5038	5039	5040	5036	5037	5038	5039	5040
Eryth ($10^6/mm^3$)		5.9	QNS	6.3	6.0	7.0	3.8	5.8	5.6	5.5	4.9
Hematocrit (%)		40	QNS	41	38	46	27	37	37	34	31
Hemogl (g/dl)		12.8	QNS	13.3	12.0	14.3	9.1	12.0	11.4	10.9	10.2

Eryth = Erythrocyte Hemogl = Hemoglobin
QNS = Quantity Not Sufficient
Data extracted from Report pages 397-401.

F. Blood Chemistry

In spite of several instances of statistically significant differences between treated and control group mean values for several parameters, the only data which are considered suggestive of a test article effect were a decrease in group mean cholesterol levels. (See Table 4)

Table 4

INDIVIDUAL ANIMAL VALUES FOR CHOLESTEROL IN RABBITS ADMINISTERED METHANEARSONIC ACID FOR 21 DAYS

Dose	Baseline					Group	Terminal					Group
mg/kg	#1	#2	#3	#4	#5	Mean	#1	#2	#3	#4	#5	Mean
MALES												
0	52	46	55	69	71	59	36	46	72	35	37	45
100	26	44	68	69	37	49	22	36	33	41	27	32
300	59	48	64	136	70	75	35	28	13	22	36	27*
1,000	177	75	84	50	72	92	23	24	35	12	15	21**
FEMALES												
0	38	88	153	46	52	77	26	36	22	33	36	31
100	84	57	71	39	80	66	33	32	24	10	19	24
300	70	72	75	61	57	67	26	27	26	9	12	20
1,000	49	76	38	62	85	62	29	55	22	46	37	38

NOTE: Cholesterol values = mg/dl
= rabbit numbers 1, 2, 3, 4 and 5
Historical Control Range = 53-117 (Report page 287)
Data extracted from Report pages 289-328.

Because of the individual values presented in Table 4, it is felt that a definitive test article effect cannot be made.

G. Urinalysis

There were no parameters which were considered to be affected by the administration of Methanearsonic acid.

H. Pathology

1. GROSS - There were no gross pathology findings at necropsy which appeared to be test article related.

2. ORGAN WEIGHTS - A statistically significant ($p<0.05$ or 0.01) increase in female kidney to body weight ratio at 100 mg/kg and for female liver to body weight ratios at 100 and 1,000 mg/kg was observed. These data are not considered to be definitive regarding a test article effect.

3. MICROSCOPIC - The pathology report stated, "When compared to the control rabbits, the administration of 1,000 mg/kg of Methanearsonic acid, as used in this study, did not cause any dermal irritation at the treatment

site nor any systemic toxicity in the full screen of tissues evaluated."

- -

REVIEWER'S COMMENTS

1. The following report pages were illegible and must be replaced: clinical chemistry, pages 329-360; hematology, pages 402-432; and urine chemistry, pages 475-482.

2. Analytical data regarding test article purity and stability need to be provided by the Registrant.

Classification of this Report: This Report is considered to be Core Supplementary. Submission of the replacement pages and analytical data would allow this Report to be upgraded to Core Minimum, provided they are acceptable to the Agency.

The Reviewer has no other comments regarding the Methods and Materials section of the Report.

Brief statements regarding statistical methodology were included in the Report.

A Good Laboratory Practice Compliance statement, A Quality Assurance statement and the Quality Assurance inspection dates were included.

III. DISCUSSION

Analytical data regarding test article purity and stability need to be provided by the Sponsor.

All rabbits survived the 21-day study. There were no clinical signs nor apparent effects of test article administration on treated skin (no erythema or edema).

Although there were slight differences between treated and control groups regarding some hematologic and blood chemistry parameters, no definitive findings were considered related to treatment. Urinalysis findings were similar for all groups.

Gross pathology, organ weights (absolute, relative to body weights or relative to brain weights) and microscopic pathology did not appear to be affected by dermal administration of methanearsonic acid.

IV. CONCLUSION

Methanearsonic acid was placed on the intact skin of the back (hair clipped) of rabbits at doses of 0 (deionized water control), 100, 300 and 1,000 mg/kg for 6 hours (occlusive dressing) 5 days/ week for 3 weeks. There did not appear to be any skin changes or other definitive effects caused by test article administration.

Systemic Toxicity No Observed Effect Level (NOEL) = 1,000 mg/kg (Limit Dose)

Systemic Toxicity Lowest Observed Effect Level (LOEL) = not attained (>1,000 mg/kg, HDT)

Dermal Toxicity No Observed Effect Level (NOEL) = 1,000 mg/kg (Limit Dose)

Dermal Toxicity Lowest Observed Effect Level (LOEL) = not attained (>1,000 mg/kg, HDT)

Classification: Core Supplementary (Registrant needs to provide replacement pages and analytical data acceptable to the Agency in order to upgrade this Report to Core Minimum).

This study does not satisfy the Guideline requirements (§82-2) for a 21-day dermal toxicity study in rabbits.

EXECUTIVE SUMMARY

014343 //

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: DEVELOPMENTAL TOXICITY – RAT
[OPPTS 870.3700 (§83-3a)]
MRID NO. 41926401

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Robert H. Ross
Signature: for D. L. Fefee
Date: MAY 2 2 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

H T Borges
Signature:
Date: MAY 2 2 2000

Robert H. Ross, M.S., Group Leader

Robert H. Ross
Signature:
Date: MAY 2 2 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

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Supplement to Tox Document 009496, review for Accession 41926401, developmental toxicity study - rat. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 8/28/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 8/31/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat

DP BARCODE: D265953
PC CODE: 013803

SUBMISSION CODE: S579557
TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (99.4% a.i.)

SYNONYM: Monosodium acid methanearsonate (MSMA)

CITATION: Schroeder, R. (1990) A teratology study in rats with methanearsonic acid. Bio/dynamics, Inc., East Millstone, NJ. Laboratory Report Number 89-3456, January 24, 1990. MRID 41926401. Unpublished.

SPONSOR: Fermenta ASC Corporation, Mentor, OH.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 41926401), methanearsonic acid (99.73% a.i.; Batch No. 107/84) was administered in deionized water by gavage to 25 mated female CD® (Sprague-Dawley) rats per group at doses of 0, 10, 100, or 500 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed and necropsied. Weights of uteri and ovaries, the number of corpora lutea, and the numbers and locations of live and dead fetuses, early and late resorptions, and implantation sites were recorded. All fetuses were weighed, sexed, and examined externally. Approximately one-half of each litter was evaluated for visceral abnormalities by microdissection, then decapitated and the heads fixed in Bouin's solution for subsequent evaluation. The remaining one-half of each litter was processed for skeletal examination.

One rat in the 500 mg/kg/day group died on GD 11 after exhibiting ano-genital staining on GD 10 and weight loss (63 g) during the GD 6-11 interval. At 500 mg/kg/day, there was a slightly increased total incidence of ano-genital staining (7 vs 0) and soft stools (7 vs 0) during treatment.

Mean body weight gain was significantly decreased at the 100 and 500 mg/kg/day dose levels during GD 12-16 (58 and 77% of controls, respectively; $p<0.05$ or $p<0.01$) and GD 6-16 (60 and 83% of controls, respectively; $p<0.01$). Additionally, rats of the 500 mg/kg/day exhibited a mean weight loss during GD 6-9 (-3 g vs. +10 g for controls; $p<0.01$). A dose-dependant decrease in gravid uterine weight was observed (80 g, 76 g, 75 g, and 74 g for control, 10, 100,

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and 500 mg/kg/day, respectively). This decrease in gravid uterine weight is corelated with the decreased mean fetal body weight observed at 500 mg/kg/day. Group mean food consumption in the 100 and 500 mg/kg/day groups was decreased compared to control at one or more intervals during treatment. **The maternal toxicity LOAEL is 100 mg/kg/day, based on decreased body weight gain and food consumption, and the maternal toxicity NOAEL is 10 mg/kg/day.**

There were no differences between the control and treated groups for number of corpora lutea per dam, number of implantation sites per dam, preimplantation loss, viable fetuses per litter, total resorptions or number of litters with resorptions. At 500 mg/kg/day, mean fetal weight was decreased (9% less than controls; $p<0.01$). There were no treatment related effects on external or visceral malformations or variations. There were also no treatment related effects on skeletal observations. **The developmental toxicity LOAEL is 500 mg/kg/day, based on decreased mean fetal body weight. The developmental toxicity NOAEL is 100 mg/kg/day.**

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

Attachment 1

The following attachment is not available electronically
See the file copy.

014343 11A
CASWELL FILE

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

003496

MAY 11 1992

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Monosodium Acid Methanearsonate (Methanearsonic Acid) - Developmental Toxicity Study in Rats (§82-3A)

Caswell No.: 582 MRID No.: 419264-01
HED Project No.: 1-1990 Chemical No.: 013803
Identification No.: 013803-042519

FROM: Alan C. Levy, Ph.D., Toxicologist Alan C. Levy
Review Section IV, Toxicology Branch II 4-30-92
Health Effects Division (H7509C)

TO: Barbara Briscoe/Betty Crompton, PM 51
Special Review and Reregistration Division (H7508W)

THRU: Elizabeth A. Doyle, Ph.D., Section Head E.A. Doyle
Review Section IV, Toxicology Branch II 5/5/92
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II M van Gemert 5/6/92
Health Effects Division (H7509C)

REQUEST: Review a Developmental Toxicity study in rats with Methanearsonic acid (Monosodium acid Methanearsonate)

CONCLUSIONS:

Methanearsonic acid was administered by gavage to pregnant rats at doses of 0, 10, 100 and 500 mg/kg on gestation days 6 through 15. The results were as follows:

10 mg/kg - maternal = none
fetal = none

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100 mg/kg - maternal = slight decrease in body weight gain and food consumption during the dosing period
fetal = none

500 mg/kg - maternal = a decrease in body weight gain and food consumption during dosing; anogenital staining and/or soft stools
fetal = lower group mean fetal body weights

Maternal No Observed Effect Level (NOEL) = 10 mg/kg
Maternal Lowest Observed Effect Level (LOEL) = 100 mg/kg - slight decrease in body weight gain and food consumption during dosing

Developmental No Observed Effect Level (NOEL) = 100 mg/kg
Developmental Lowest Observed Effect Level (LOEL) = 500 mg/kg - lower group mean fetal body weights.

The test article did not appear to cause any teratogenic effects.

Classification: Core Minimum

This study satisfies the Guideline requirements (§83-3A) for a developmental toxicity study in rats.

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4-30-92
Section IV, Tox. Branch II (H7509C)

Secondary reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/5/9
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity - Rat (§83-3A)

TEST MATERIAL: Methanearsonic acid (monosodium acid methanearsonate)

SYNONYMS: T-168-2, MAA, SDS-37161

Tox. Chemical No.: 582 **MRID No.:** 419264-01
HED Project No.: 1-1990 **Identification No.:** 013803-042519
Chemical No.: 013803

STUDY NUMBERS: Sponsor (Fermenta) = 89-0130
Performing Laboratory (Bio/dynamics) = 89-3456
Test Substance Analysis Laboratory (Ricerca) = 89-0130
Ricerca Document No.: 3190-89-0130-TX-000, 001,002
TS-001

SPONSOR: Fermenta ASC Corporation, Mentor, OH

TESTING FACILITY: Animal Study = Bio/dynamics Inc., East Millstone, NJ
Test Substance Analysis = Ricerca, Inc., Painesville, OH

TITLE OF REPORT: A Teratology Study in Rats with Methanearsonic Acid

AUTHORS: Bio/dynamics = Raymond E. Schroeder
Ricerca = M. Mizens and J. C. Killeen

REPORT ISSUED: Bio/dynamics = January 24, 1990
Ricerca = September 7, 1990

CONCLUSIONS:

Methanearsonic acid was administered by gavage to pregnant rats at doses of 0, 10, 100 and 500 mg/kg on gestation days 6 through 15. The results were as follows:

10 mg/kg - maternal = none
fetal = none

100 mg/kg - maternal = slight decrease in body weight gain and food consumption during the dosing period
fetal = none

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500 mg/kg - maternal = a decrease in body weight gain and food consumption during dosing; anogenital staining and/or soft stools
fetal = lower group mean fetal body weights

Maternal No Observed Effect Level (NOEL) = 10 mg/kg
Maternal Lowest Observed Effect Level (LOEL) = 100 mg/kg - slight decrease in body weight gain and food consumption during dosing

Developmental No Observed Effect Level (NOEL) = 100 mg/kg
Developmental Lowest Observed Effect Level (LOEL) = 500 mg/kg - lower group mean fetal body weights

Classification: Core Minimum

This study satisfies the Guideline requirements (§83-3A) for a developmental toxicity study in rats.

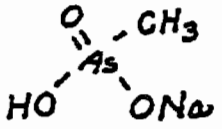
009190

I. MATERIALS, METHODS AND RESULTS

A. Test Article Description

Name: Methanearsonic acid (monosodium acid methanearsonate, T-168-2, methylarsonic acid, MAA, SDS-37161)

Formula:



Lot Number: 107/84 (Batch No.)
Purity: 99.73% (>98% Ricerca Report)
Appearance: white powder
Storage: room temperature, in the dark

Vehicle: deionized water

B. Stability of Test Article

Analyses were peformed by Ricerca, Inc. This Ricerca Report indicates that the purity was ">98% SDS-37161 (recrystallized)" (Ricerca Report page 9, Report page 457). Table 1 presents purity and stability data.

Table 1

A SUMMARY OF PURITY/STABILITY ANALYTICAL DATA OF METHANEARSONIC ACID IN DOSING SOLUTIONS FOR A RAT DEVELOPMENTAL TOXICITY STUDY

	Assay No.	1.0 mg/ml	10.0 mg/ml	50.0 mg/ml
Day 0	1	0.9	-	52.0
	2	1.0	-	51.9
Day 15	1	1.0	-	49.4
	2	1.0	-	49.6
Mix	1	1.0	10.1	48.0
	1	1.0	9.9	49.6
	2	1.0	10.0	50.4
	2	1.0	10.0	50.2
	3	1.0	9.9	49.1
	3	1.0	9.8	49.6

NOTE: control assays = <0.01 mg/ml
Data extracted from Ricerca Report Tables 2-4, pages 15-17 (These are Report pages 463-465). Report pages 450-490 contain the complete Ricerca Assay Report.

Purity and stability assay data indicate that these are acceptable.

C. Dosing

Methanearsonic acid was administered by gavage in volumes of 10 ml/kg body weight/day on gestation days 6 through 15 at doses of 0 (deionized water), 10, 100 and 500 mg/kg/day. The vehicle was deicnized water. Volumes were adjusted based on the most recent body weights. Fresh dosing solutions were prepared once before the initiation of dosing and 3 times during the dosing period. Because mating (day 0 of gestation) took place on 12 separate days, the 10 doses (gestation days 6-15) were administered to all rats in the study over a staggered period of 32 days.

D. Animals

CD® (Sprague-Dawley) rats were obtained from Charles River Laboratories, Inc., Portage, MI. At the initiation of mating, males (proven breeders) were about 23 weeks old and females were non-pregnant/nulliparous,about 10 weeks old, and had been acclimated for 23 days.

Animals were individually housed in stainless steel wire mesh suspended cages except during mating when one male was caged overnight with one female. Food and water were available ad libitum. Actual room temperature during the study was 72°F (68-82°F, out of desired range of 67-73°F 11 times). Actual room humidity during the study was 62% (50-77%, out of desired range of 30-70% on one occasion). There was a 12 hour light/dark cycle.

Animals were examined by a veterinarian before being assigned to the study. Mated females were placed in groups daily so as to keep the group mean body weights equal.

E. Mating

After a 1:1 overnight mating, vaginal smears were obtained and mating was considered to have taken place if sperm and/or a vaginal plug was observed. Day 0 of gestation was the day evidence of mating was noted. There were 25 females mated/group.

F. Observations

1. Physical

Mated females were observed A.M. and P.M. for

appearance, behavior, signs of toxicity, moribundity and mortality. Each was also given a detailed physical examination on gestation days 0, 6-15 and 20.

The only death was a 500 mg/kg rat (No. 4586) which died on gestation day 11 after having received 6 doses. The female was not pregnant and there did not appear to be an intubation injury. There was staining of the skin/fur in the ano-genital region on gestation day 10 and there was a loss of 63 g of body weight during days 6-11.

There were no clinical signs attributed to test article administration in the 10 or 100 mg/kg groups. At 500 mg/kg, 3 rats had ano-genital skin/fur staining at one or more intervals during treatment. Five rats from this group (including 1/3 with staining) were noted to have soft stools at one or more intervals during treatment or post-treatment. As neither the staining nor soft stools were reported in the controls, 10 or 100 mg/kg animals, the Report stated that this "low incidence" of stains and/or soft stools was, "... suggestive of a treatment related response."

2. Body Weights

Weights were recorded once during the acclimation period as well as on gestation days 0, 6, 9, 12, 16 and 20. Body weights and weight gains are presented in Table 2.

There was not a statistically significant nor a strong indication of a biologically significant difference in group mean body weights at any weighing interval regarding any of the groups. Also, group mean corrected body weights (final weight minus gravid uterine weight) appeared to be similar for all groups.

For body weight gains, the 10 mg/kg group means were similar to controls for all time periods. At 100 mg/kg, there was a lower gain ($p<0.01$ or 0.05) for the 12-16 and 6-16 day periods.

At 500 mg/kg, there was a slight numerical group mean loss (3 g) for days 6-9 (beginning of dosing) compared with gains of 9-10 g for the other 3 groups ($p<0.01$ versus control). During days 9-12, this high dose group mean weight gain was 20 g compared with 16, 12 and 13 for the 0, 10 and 100 mg/kg values. All 4 groups had similar mean gains for days 16-20 (61-67 g). For the entire dosing period (days 6-16), there was a lower group mean gain ($p<0.01$) at 500 mg/kg (31 g) compared with the control gain (52 g).

Methanearsonic acid at 500 mg/kg appears to have had an effect on body weight gain during the period of dosing with an overall gain during pregnancy (days 0-20) 11% less than the control group (146 versus 130 g). The two lower dose groups gained 136 g. The statiscically significant lower gain at 100 mg/kg during days 12-16 and 6-16 is suggestive of a test article effect.

Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS DURING GESTATION IN A RAT TERATOLOGY STUDY WITH METHANEARSONIC ACID

Day mg/kg =	0	10	100	500
BODY WEIGHT (G)				
0	229	227	225	230
6	258	257	255	262
9	268	266	265	258
12	284	278	278	278
16	310	302	298	293
20	375	363	361	360
CORRECTED BODY WEIGHT (day 20 weight minus gravid uterine weight)				
Body Weight (g)	295	287	286	286
Uterine Weight (g)	80	76	75	74
BODY WEIGHT GAIN (g)				
0-6	29	30	30	32
6-9	10	9	10	-3**
9-12	16	12	13	20
12-16	26	24	20**	15**
16-20	64	61	63	67
6-16	52	45	43*	31**
0-20	146	136	136	130

NOTE: The number of pregnant females/group was (mg/kg) : 0 = 23, 10 = 25, 100 = 24 and 500 = 23. Data from one 100 mg/kg female were excluded due to incorrect body weight recording which resulted in an over-dose on gestation days 9-11.
Statistical Significance: * = $p<0.05$; ** = $p<0.01$
Data extracted or calculated from Report Appendices C, D and E, pages 105, 110 and 116.

3. Food Consumption

Data for this parameter were recorded for the following periods (days): 0-6, 6-9, 9-12, 12-16 and 16-20.

No adverse effect was noted for animals receiving 10 mg/kg.

Group mean food consumption (g/kg/day and g/rat/day) was less than the control value for one or more intervals during dosing at 100 and 500 mg/kg. This appears to be consistent with the body weight gain values. At 500 mg/kg, the days 16-20 value (g/rat/day) was essentially equal to the control (29 versus 28 g).

G. Reproductive Data

Complete postmortem examinations were performed on all mated females. Tissues with lesions were preserved.

The animals were sacrificed by exsanguination under ether anesthesia on gestation day 20. The intact uterus (ovaries attached) was weighed and the number and location of the following recorded for each uterine horn: live fetuses, dead fetuses, late resorptions, early resorptions and implantation sites. If no implants were observed, the uterus was stained with ammonium sulfide. The animal was considered non-pregnant if no post-staining implants were observed. The ovaries were examined for the number of corpora lutea.

Table 3 presents a summary of the reproductive data.

Table 3

A SUMMARY OF REPRODUCTIVE DATA FROM A RAT DEVELOPMENTAL TOXICITY STUDY WITH METHANEARSONIC ACID

Parameter mg/kg =	0	10	100	500
Females Mated - No.	25	25	25	25
Pregnant - No.	23	25	24a	23
Litters with Viable Fetuses - No.	23	25	24	23
Female Mortality - No.	0	0	0	1
Corpora Lutea - group mean	16.2	15.9	16.0	16.3
Implantation Sites - group mean ..	15.3	15.3	14.8	15.3
Viable Fetuses - mean litter size	14.4	14.3	14.0	14.7
Dead Fetuses	0	0	0	
Resorptions - total	19	24	20	12
Litters with Resorptions	12	14	12	10
Fetal Body Weight - group mean ...	3.4	3.3	3.3	3.1**
Males	3.5	3.4	3.4	3.2**
Females	3.3	3.2	3.2	3.0**

a = One of 25 excluded due to incorrect weighing which resulted in overdosing
Statistical Significance: * = p<0.05; ** = p<0.01
Data extracted from Report Appendix G, page 131.

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Two control and two 500 mg/kg females were not pregnant. There was no apparent test article effect on any of the uterine or ovarian parameters examined.

Male, female and combined sex fetal weight data indicated that, at 500 mg/kg only, there was a statistically significant ($p<0.01$) group mean lower weight when compared with control data. Therefore, this dose appeared to have a fetotoxic effect.

H. Fetal Evaluations

1. External/Visceral/Head

Each fetus was subjected to the following: gross examination for external changes, weighing and sexing.

About half of the fetuses in each litter (alternating within a litter) were evaluated for visceral changes (microdissection). These were decapitated and the heads placed in Bouin's for evaluation. After internal examination, the fetuses were eviscerated, placed in cassettes and stored in 70% ethanol.

The only external findings during an examination of all fetuses from the four groups were the following:

10 mg/kg: One fetus had an umbilical hernia. A second fetus had edema of the cervical and thoracic regions, absence of ear folds, small eye bulges, micromelia with absence of digits (fore- and hindlimbs), cleft in abdominal musculature and absence of anal opening as well as genital tubercle. These findings are not considered to be treatment related.

All other external, visceral or head findings were similar in all groups, were of a relatively minor nature or were in such few specimens that they did not appear to be the result of test article administration.

2. Skeletal

The remaining fetuses from each litter were sacrificed by ether, eviscerated and processed for skeletal examination using Alizarin Red S. They were examined under a dissecting microscope and then stored in glycerin.

Skeletal examination of fetuses did not reveal any indication that the administration of the test article had any effects.

The Reviewer has no comments regarding the methods and materials of this Report.

Historical control data were included in the Report (Report pages 437-445).

Detailed statistical analysis procedures were described in the report.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement and a list of Quality Assurance inspections were included in the report.

The Registrant stated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the results of this study and that the study neither meets nor exceeds any of the applicable criteria. This Reviewer agrees.

II. DISCUSSION

Body weight gain was reduced during dosing at 500 mg/kg. There was also the possibility of a lower weight gain at the 100 mg/kg dose during this same period (days 6-16 of gestation). Food consumption was reduced at 100 and 500 mg/kg during dosing, essentially paralleling the decreases in body weight gain.

At 500 mg/kg, there was staining of the skin/fur in the anogenital area and/or an increase in the incidence of soft stools.

There appeared to be a decrease in group mean fetal body weights at the high dose of 500 mg/kg.

None of the treated groups showed any external, visceral or skeletal changes which were considered to be related to test article administration.

III. CONCLUSION

Methanearsonic acid was administered by gavage to pregnant rats at doses of 0, 10, 100 and 500 mg/kg on gestation days 6 through 15. The results were as follows:

10 mg/kg - maternal = none
fetal = none

100 mg/kg - maternal = slight decrease in body weight gain and food consumption during the dosing period
fetal = none

500 mg/kg - maternal = a decrease in body weight gain and food consumption during dosing; anogenital staining and/or soft stools
fetal = lower group mean fetal body weights

Maternal No Observed Effect Level (NOEL) = 10 mg/kg
Maternal Lowest Observed Effect Level (LOEL) = 100 mg/kg - slight decrease in body weight gain and food consumption during dosing

Developmental No Observed Effect Level (NOEL) = 100 mg/kg
Developmental Lowest Observed Effect Level (LOEL) = 500 mg/kg - lower group mean fetal body weights

Classification: Core Minimum

This study satisfies the Guideline Requirements (§83-3.B) for a developmental toxicity study in rats.

Page_____ is not included in this copy.

Pages 172 through 187 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

EXECUTIVE SUMMARY

014343 (1

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: METABOLISM - RAT
[OPPTS 870.7485 (§85-1)]
MRID NO. 42010501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: HT Borges
Date: MAY 22 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

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EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: METABOLISM - RAT
[OPPTS 870.7485 (§85-1)]
MRID NO. 42010501

Prepared for

Health Effects Division
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1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M. Signature:_____
Date: _____

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

Supplement to Tox Document 009374, review for Accession 42010501, metabolism study - rat. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 9/7/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 9/12/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat [OPPTS 870.7485 (§85-1)]

DP BARCODE: D265953 SUBMISSION CODE: S579557
PC CODE: 013803 TOX CHEM NO: 582

TEST MATERIAL: [^{14}C-methyl] Monosodium methanearsonate (radiochemical purity ≥99.4%); Monosodium methanearsonate (Lot No. ASC 66878-0101 chemical purity = 100.9%; Lot No. 66878-0102 chemical purity = 100.2%)

SYNONYMS: MSMA

CITATION: Wells-Gibson, N., J. Marsh, G. Krautter (1991) Absorption, distribution and elimination of [^{14}C-methyl]MSMA in the rat. PTRL East, Inc., 3945 Simpson Lane, Richmond, KY 40475. Laboratory Report Number PTRL Report No. 1344, August 30, 1991. MRID 42010501. Unpublished.

SPONSOR: MAA (MSMA/DSMA) Research Task Force 3, Luxembourg Industries (PAMOL), Ltd., 27 Hamered Street, P.O. Box 13, Tel Aviv 61000, Israel.

EXECUTIVE SUMMARY: This study was designed to identify parent compound and metabolites in the urine and feces over a 7-day period, in expired CO_2 over a 1-day period, and in tissue 7 days after treatment (MRID 42010501).

In this metabolism study, groups Sprague-Dawley CD® (Crl:CDBR) rats were given a [^{14}C-methyl]-monosodium methanearsonate (≥99.4% a.i.; Lot/Batch No. ICN CFO 2289, GPS/2/79/1) in water at concentrations of 0, 5.0, or 200.0 mg/kg according to the following five different dose groups: 1) In the vehicle control group, 3 rats/sex were dosed with water by gavage; 2) Single low dose group: 5 rat/sex were given a single radiolabeled gavage dose of 5.0 mg/kg; 3) Single high dose group: 5 rats/sex were given a single radiolabeled gavage dose of 200 mg/kg; 4) Consecutive dosing group: 5 rats/sex were dosed by gavage for 14 consecutive days with unlabelled MSMA at 5.0 mg/kg/day followed by a single radiolabelled dose of MSMA at MSMA; 5) I.V. dose group: 5 rats/sex were given a single radiolabeled i.v. dose of 5.0 mg/kg. Expired CO_2 was sampled at 0.5 and 1 day post-dosing, and urine and fecal samples were taken at intervals up to 7 days post-dosing, whereupon the rats were terminated. Tissue samples (blood, bone, brain, fat, heart, kidney, liver, lungs, muscle, ovary, skin, spleen, testis, uterus, and residual carcass) were taken at termination.

There were no treatment related deaths or clinical signs.

At 0.5 day following treatment, 27.5-40% and 12-34% of the administered dose were measured in the urine and feces, respectively, in rats receiving gavage doses, including the low single dose group, high single dose group and consecuting dosing group. Urine accounted for 33-42% of the excretion in the oral 5 and 200 mg/kg treatment groups. Fecal elimination accounted for 38-58% of the radiolabel in the oral 5 and 200 mg/kg groups. By day 1, 69%-88% of the administered dose was excreted in the urine and feces combined. In rats dosed by gavage, at day 3, 83% to 97% of the administered dose was excreted in the urine and feces. Less than 0.5% of the radiolabel was recovered as CO_2 in all groups. Male and females rats excreted radiolabelled MSMA at similar rates.

At 0.5 day following treatment, 82% of the administered dose in both sexes was excreted in the urine of rats dosed by i.v. injection. At day 1 following treatment, 93% and 91% of the administered dose was excreted predominately in the urine; males excreted 4% of the administered dose in the feces. Less than 0.5% of the radiolabel was recovered as CO_2 in all groups.

Tissue levels of radiolabel ranged from 6-14% in all oral treatment groups and ~3% in the 5 mg/kg iv treatment group 7 days after treatment. The blood (2-4% in gavage dose groups) contained the highest concentrations of radiolabel followed by the liver, kidney, and spleen which contained < 1.0% of the administered dose. Total recovery of administered radioactivity over the 7-day period for all treated groups was ~98.2% of the administered dose.

Analysis of fecal and urinary samples by HPLC and TLC revealed that the radioactivity of all preparative fractions was associated with parent compound and two unknown metabolites. The major product excreted in both urine and feces was unchanged parent, accounting for 80-97% of the administered dose. Unknown metabolite A was detected in the urine and feces of the low-dose groups (6.7/6.1, 1.8/2.6, and 3.7/3.7% of the administered dose for males/females of the single 5.0 mg/kg po, 5.0 mg/kg po following pretreatment, and 5.0 mg/kg iv groups, respectively). Unknown metabolite B was detected in the urine and feces of the group that received 5.0 mg/kg following pretreatment at 0.7% of the administered dose for both males and females.

This study is classified as **Acceptable/ Guideline** and satisfies the requirements for a metabolism study in rats [OPPTS: 870.7485 (§85-1)].

Attachment 1

The following attachment is not available electronically
See the file copy.

014343

(12)

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

009374

MAR 13 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

Subject: Review of Toxicology Studies with Methanearsonic Acid/Methanearsonic acid, monosodium salt to support reregistration of the test substance. (Toxchem Number 582, HED Project No. 2-0879; Barcode number: D172561)

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 3/15/92
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Barbara Briscoe PM (51)/Betty Crompton PM Team Reviewer
Special Review and Reregistration Division
HED (H7508W)

THRU: Elizabeth Doyle, Ph.D., Section Head E.A. Doyle 3/17/92
Tox. Section II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Tox. Branch II
HED (H7509C) M. van Gemert 3/18/92

ACTION REQUESTED: Review of toxicology study for reregistration requirements for MRID 420105-01, Guideline 85-1.

Study Summarized

MRID 420105-01, Metabolism Study (85-1); Core - guideline

Sprague-Dawley CD rats were treated by oral gavage with a single dose of [^{14}C-methyl]MSMA at doses of 0 (control), 5 or 200 mg/kg, 5 mg/kq MSMA orally for 14 consecutive days followed by a single oral dose of 5 mg/kg of [^{14}C-methyl]MSMA or a single i.v. injection of 5 mg/kg of [^{14}C-methyl]MSMA.

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[^{14}C-methyl]MSMA was excreted in the urine and feces 24-48 hours after treatment, primarily as unchanged parent. The mean total radiocarbon recovered as $^{14}CO_2$ accounted for ≤0.5% of the administered dose in all treated groups.

During the 7 days following dosing, the mean total recovery of radiocarbon in urine and feces was 91.3% for males and 88.8% for females. Specifically, the oral dose group (both sexes) excreted a mean of 41% of the administered dose in the urine and 48% in the feces; the i.v. group excreted 91% of the radiocarbon in the urine. Comparison of the radiocarbon excreted in the urine by the i.v. dosed rats with that excreted by the orally dosed rats indicated that approximately half of the oral dose was absorbed by the low and consecutive dose groups and somewhat less by the high dose group.

A mean of 10, 6, 13 and 3% of the administered dose remained, respectively, in the tissues and carcass at the 5 mg/kg, 200 mg/kg, consecutive and i.v infusion dose groups. In all groups, the largest fraction of the bound radiocarbon was accounted for in the blood; other organs contained lesser amounts.

Total mean recovery of radiocarbon in all treated groups was 98.2% of the administered dose.

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Reviewed by Steven L. Malish, Ph.D. S.L. Malish 3/16/92
Tox Branch II, Section IV (7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 3/17/92
Tox. Branch II, Section IV (H7509C)

DATA EVALUATION REPORT

STUDY TITLE: Metabolism Study (85-1)

MRID NO.: 420105-01

TEST MATERIAL: [^{14}C-methyl]monosodium methanearsonate; monosodium methanearsonate

SYNONYM: [^{14}C-methyl]MSMA; MSMA

SPONSOR: MAA(MSMA/DSMA)Research Task Force 3
Luxembourg Industries (PAMOL), Ltd.
27 Hamered Street
P.O. Box 13
Tel Aviv 61000, Israel

LABORATORY: PTRL East, Inc.
3945 Simpson Lane
Richmond, Kentucky 40475

REPORT NO.: PTRL Report No. 1344

REPORT TITLE: Absorption, Distribution and Elimination of [^{14}C-methyl]MSMA in the Rat

AUTHORS: N. Wells-Gibson, B.S., J. D. Marsh, M.S., G. R. Krautter, M.S.

REPORT ISSUED: August 30, 1991

CONCLUSIONS:

Sprague-Dawley CD rats were treated by oral gavage with a single dose of [^{14}C-methyl]MSMA at doses of 0 (control), 5 or 200 mg/kg, 5 mg/kg MSMA orally for 14 consecutive days followed by a single oral dose of 5 mg/kg of [^{14}C-methyl]MSMA or a single i.v. injection of 5 mg/kg of [^{14}C-methyl]MSMA.

[^{14}C-methyl]MSMA was excreted in the urine and feces 24-48 hours after treatment, primarily as unchanged parent. The mean total radiocarbon recovered as $^{14}CO_2$ accounted for ≤ 0.5% of the administered dose in all treated groups.

During the 7 days following dosing, the mean total recovery of radiocarbon in urine and feces was 91.3% for males and 88.8% for females. Specifically, the oral dose group (both sexes) excreted a

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mean of 41% of the administered dose in the urine and 48% in the feces; the i.v. group excreted 91% of the radiocarbon in the urine. Comparison of the radiocarbon excreted in the urine by the i.v. dosed rats with that excreted by the orally dosed rats indicated that approximately half of the oral dose was absorbed by the low and consecutive dosed animals and somewhat less by the high dosed animals.

A mean of 10, 6, 13 and 3% of the administered dose remained, respectively, in the tissues and carcass at the 5 mg/kg, 200 mg/kg, consecutive and i.v infusion dose groups. In all groups, the largest fraction of the bound radiocarbon was accounted for in the blood; other organs contained lesser amounts.

Total mean recovery of radiocarbon in all treated groups was 98.2% of the administered dose.

CLASSIFICATION: Core: guideline

This study satisfies the guideline requirements (85-1) for a metabolism study.

TEST MATERIAL:	Chemical:	[^{14}C-methyl]monosodium methanearsonate (labeled); monosodium methanearsonate (unlabeled)
	Lot:	labeled: ICN CFO 2289, GPS/2/79/1, PTRL No. 457-3 (≥99.4% purity); unlabeled: ASC 66878-0[illegible], PTRL No. 468-9 (100.9% purity); ASC 66878-0102; PTRL No.481-35 (100.2% purity).
	Spec. Act:	2.4 mCi/mM
	Stability:	stable
	Storage:	-20°C.

TEST ANIMALS:	Species:	rat
	Strain:	Sprague-Dawley CD®(Crl:CDBR)
	Sex:	male/female
	Groups:	4 treated groups of 5 animals/sex, 1 control group of 3 animals/sex
	Age:	6 - 10 weeks at initiation
	Weight:	200 - 250 gms at initiation
	Source:	Portage MI facility of Charles River Labs, Inc. Wilmington, MA.

Quality Assurance - A quality assurance statement was issued.

MATERIALS AND METHODS:

Animals were acclimated for at least 7 days before being placed on

test. Certified Rodent Chow #5002 (Purina Mills, Inc.) and water was provided ad libitum. Animals in each dose group were assigned by random numbers in ascending order.

Study Design

Rats were treated by oral gavage with a single dose of [^{14}C-methyl]MSMA at 0 (control), 5 or 200 mg/kg, 5 mg/kg MSMA orally for 14 consecutive days followed by a single oral dose of 5 mg/kg of [^{14}C-methyl]MSMA or a single dose of 5 mg/kg of [^{14}C-methyl]MSMA by i.v. injection.

Four (4) treated groups consisted of 5 animals/sex. The vehicle control group consisted of 3 animals/sex. Treatment groups were placed on test one group at a time and were dosed by oral intubation (feeding needle) or by i.v. injection of the test substance into the femoral vein (Table 1).

Immediately following the dose of the radiolabeled test substance or control vehicle, the animals were transferred to individual glass metabolism cages designed to separate and collect urine, feces and expired air (for $^{14}CO_2$) and to quantify feed and water consumption.

Table 1

Dose Group Treatments[1]

	Group	Dose[2] (mg/kg)	No. of Rats/Sex	Route
1.	Control	Vehicle	3 M/F	oral intubation
2.	Single Oral	5.0	5 "	oral intubation
3.	Single Oral	200.0	5 "	oral intubation
4.	Consecutive[3]	5.0	5 "	oral intubation
5.	Single i.v.	5.0	5 "	i.v.

[1]Adapted from original report p. 16.
[2]The amount of vehicle (water) received by each rat was approximately 2.5 - 4.5 ml/kg.
[3]Rats received a single 5.0 mg/kg oral dose of [^{14}C-methyl]MSMA within 24 hours after pre-treatment with 14 consecutive oral doses of unlabeled MSMA given at 5.0 mg/kg/day.

In all treated groups, the excretion of radiocarbon in feces and urine was sampled at 0.5 and 1 thru 7 days postdose. Carbon dioxide was sampled at 0.5 and 1 day postdose.

At the time of sacrifice, 7 days after treatment with [^{14}C-methyl]MSMA or vehicle control, animals were anesthetized and exsanguinated by aortic puncture. Residual radiocarbon levels were

quantified by radioassay in the blood, bone, brain, fat (visceral), heart, kidney, liver, lungs, muscle (thigh), ovary, skin (clipped), spleen, testis, uterus and the residual carcass.

Combustion Analysis and Radioassay:

Urine, feces, blood and tissue samples were combusted to carbon dioxide, water and inorganic ash using a sample oxidizer. The radiocarbon from the combustion products were quantitated by liquid scintillation spectrometry. The expired air $^{14}CO_2$ was similarly assayed.

Statistical Analysis:

Mean and standard deviations were calculated.

Urine and Feces Sample Compositing for Metabolite Characterization

After the material balance from each definitive dose group was established, the urine and feces from each sex of each group was composited for metabolite characterization.

Extraction of Feces and Blood

Composited feces samples from each dose group/sex were extracted in phosphate buffer followed by extraction in n-hexane. The small amount of unextracted solids were then hydrolyzed by refluxing with hydrochloric acid. Extracts were quantitated by direct radioassay and analyzed for metabolites by HPLC.

Composited whole blood samples from the 200 mg/kg dose males and females were extracted with acetonitrile followed by water extraction, quantitated by direct radioassay and analyzed for metabolites by HPLC.

High Performance Liquid Chromatography (HPLC)
Whole rat urine, feces and blood extracts were analyzed for metabolites by HPLC.

Thin Layer Chromatography (TLC)

Metabolite identification in the whole urine and feces extracts were confirmed by thin layer chromatography.

RESULTS AND DISCUSSIONS:

Animal Observations

There were no signs of toxicity observed in any of the treated groups during the in vivo portion of the study.

Material Balance Studies

[^{14}C-methyl]MSMA was readily excreted in the urine and feces of all dose groups within 24-48 hours after treatment (Tables 2 thru 5). The mean total radiocarbon recovered as $^{14}CO_2$ accounted for ≤ 0.5% of the administered dose (Tables 2 thru 5).

For most of the oral dose groups (5 and 200 mg/kg and consecutive dosed females) mean total radiocarbon excreted in the feces was slightly higher than that excreted in the urine. The consecutive dosed group males excreted slightly more radiocarbon in the urine. In contrast, the i.v. dosed group excreted the majority of the radiocarbon in the urine (Tables 2 thru 6).

When comparing the amounts of radiocarbon excreted in the urine versus the feces, excretion patterns were similar between the oral 5 mg/kg and consecutive dose groups. However, when comparing the excretion patterns of the 5 mg/kg dose to the 200 mg/kg dose, the 200 mg/kg animals excreted approximately 10% more of the administered dose in the feces. This probably reflects less complete absorption at 200 mg/kg than at 5 mg/kg. In contrast, virtually all of the injected radiocarbon was excreted in the urine (Table 2 thru 6)

The total recovery of radiocarbon in urine, feces, expired air and tissues for all treated groups was 98.2% of the administered dose. Specifically, mean total recovery for the 5 and 200 mg/kg, consecutive and i.v. dosed group was 96.5, 101.1, 97.5 and 97.8%, respectively (Tables 2 thru 5).

Table 2

Cumulative Material Balance Summary for Rats Receiving a 5.0 mg/kg Oral Dose of [^{14}C-methyl]MSMA[1]

Day	Urine M/F %	Feces M/F %	CO_2 M/F %	Tissue M/F %	Total M/F %
0.5	34.8/34.6	25.8/17.1	0.5/0.4	--	61.1/52.2
1	39.9/40.0	43.1/40.3	0.7/0.6	--	83.8/81.0
2	40.8/41.0	45.3/43.8	"	--	86.8/85.4
3	41.1/41.1	45.4/43.9	"	--	87.2/85.6
7	41.6/41.6	45.4/43.9	"	9.3/9.9	97.0/96.0

[1]Adapted from the original report, p. 55 thru 58.
[2]Mean values, statistical analyses not performed.

Table 3

Cumulative Material Balance Summary for Rats Receiving a Single 200 mg/kg Oral Dose of [^{14}C-methyl]MSMA[1,2]

Day	Urine M/F %	Feces M/F %	CO_2 M/F %	Tissue M/F %	Total M/F %
0.5	27.5/20.5	30.0/16.8	0.5/0.3	--	58.0/37.6
1	37.6/30.5	49.8/43.9	0.5/0.4	--	87.9/74.8
2	39.2/33.0	56.1/56.5	"	--	95.8/89.9
3	39.5/33.4	56.5/57.8	"	--	96.6/91.6
7	40.3/33.7	57.1/58.0	"	5.9/6.3	103.7/98.4

[1]Adapted from the original report, p. 59 thru 62.
[2]Mean values, statistical analyses not performed.

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Table 4

Cumulative Material Balance Summary for Rats Receiving 14 Consecutive Doses of 5.0 mg/kg MSMA Followed by a Single Oral Dose of 5.0 mg/kg [^{14}C-methyl]MSMA[1,2]

Day	Urine M/F %	Feces M/F %	CO_2 M/F %	Tissue M/F %	Total M/F %
0.5	40.5/30.4	25.7/12.1	0.4/0.4	--	66.6/42.9
1	45.4/35.4	35.0/33.5	0.4/0.4	--	80.8/69.3
2	46.3/37.2	38.1/44.8	"	--	84.8/82.4
3	45.6/37.7	38.2/44.8	"	--	85.2/82.9
7	47.4/38.3	38.3/44.8	"	11.9/13.6	97.9/97.1

[1]Adapted from the original report, p. 63 thru 66.
[2]Mean values, statistical analyses not performed.

Table 5

Cumulative Material Balance Summary for Rats Receiving a Single I.V. Infusion Dose of 5.0 mg/kg [^{14}C-methyl]MSMA[1,2,3]

Day	Urine M/F %	Feces M/F %	Tissue M/F %	Total M/F %
0.5	82.6/81.9	3.1/0.0	--	85.6/81.9
1	88.6/90.8	4.4/0.4	--	93.0/91.2
2	89.4/92.3	4.9/1.5	--	94.3/93.8
3	89.7/92.6	5.1/1.7	--	94.7/94.3
7	89.9/92.8	5.3/2.1	2.7/2.9	97.9/97.7

[1]Adapted from the original report, p. 67 thru 70.
[2]Mean values, statistical analyses not performed.
[3]No respiratory $^{14}CO_2$ recovered.

After 7 days post-treatment, the mean cumulative urinary excretion of administered radiocarbon for male and female animals at 5.0 mg/kg was 41.6%; at 200 mg/kg 40.3% and 33.7%; at the consecutive dose 47.4 and 38.3% and in the i.v. dose 89.9 and 92.8%, respectively. The mean total recovery of radiocarbon in urine and feces was 91.3% for males and 88.8% for females (Table 6).

Table 6

Urine and Feces Cumulative Excretion Pattern [^{14}C-methyl]MSMA 7 Days After Dosing[1,2]

Dose	Urine M/F %	Feces M/F %	Total M/F %
Single (5 mg/kg)	41.6/41.6	45.4/43.9	87.0/85.5
Single (200 mg/kg)	40.3/33.7	57.1/58.0	97.4/91.7
Consecutive[3]	47.4/38.3	38.3/44.8	85.7/83.1
Single i.v.	89.9/92.8	5.3/2.1	95.2/94.9

[1]Adapted from the original report. p. 58, 62, 66 and 70.
[2]Mean values, statistical analyses not performed.
[3]Rats received a single 5.0 mg/kg oral dose of [^{14}C-methyl]MSMA within 24 hours after pre-treatment with 14 consecutive oral doses of unlabeled MSMA given at 5.0 mg/kg/day.

Tissue levels

All animals were sacrificed 7 days after treatment with radiolabeled [^{14}C-methyl]MSMA or vehicle (control group) and tissue residues quantitated. The highest level of radioactivity were found in the blood. The blood contained (as a percentage in male/female) 3.2M/3.7F at 5 mg/kg; 2.3M/2.4F at 200 mg/kg; 3.7M/4.1F in the consecutive dose and 0.9% in both males and females in the i.v. infusion dose level. Lesser amounts were found in the tissues [e.g. up to 0.12% (liver), 0.24% (kidney) and 0.38% (spleen)].

Between 9.3M/9.9F; 5.9M/ 6.3F; 11.9M/13.6F and 2.7M/2.9F percent of the administered dose remained in tissues and the carcass of the 5.0 mg/kg, 200 mg/kg, consecutive and i.v. infusion groups, respectively (Tables 2 thru 5).

HPLC Metabolite Profiles of Rat Urine and Feces

A total of 3 metabolites were observed in the urine and/or feces samples. The major product excreted in both urine and feces was unchanged parent, accounting for 79.7 to 97.4% of the administered dose. A minor unknown metabolite product (unknown A was detected in the urine and feces of the low dose groups which accounted for 1.8 to 6.7% of the dose. This unknown metabolite was not excreted by the high dose group animals. A second unknown minor metabolite (unknown B) was detected in trace amounts (0.7%) in the urine of consecutively dosed animals (Table 7).

Table 7

Quantitation of Metabolites in Rats Dosed with [^{14}C-methyl]MSMA[1]

	Recovery as Percent of Dose							
	I.V. 5.0 mg/kg		Oral 5.0 mg/kg		Oral 200.0 mg/kg		Oral Consecutive plus 5.0 mg/kg[2]	
Metabolite	M	F	M	F	M	F	M	F
Unknown A	3.7	3.7	6.7	6.1	0.0	0.0	1.8	2.5
Unknown B	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7
MSMA	92.8	91.5	80.3	79.7	97.4	91.7	83.2	79.7
Total	94.9	95.2	87.0	85.8	97.4	91.7	85.7	83.0

[1]Data from original report, p. 82.
[2]Rats received a single 5.0 mg/kg oral dose of [^{14}C-methyl]MSMA within 24 hours after pre-treatment with 14 consecutive oral doses of unlabeled MSMA given at 5.0 mg/kg/day.

Confirmatory TLC of Rat Urine and Feces Metabolites

All urine and fecal samples analyzed by HPLC were also analyzed by TLC. Quantitation of the sample spots that co-migrated with [^{14}C-methyl]MSMA as a percent of the administered dose, correlated closely with the values obtained from HPLC analysis. The results confirmed that the identity of the major metabolite in urine and feces was [^{14}C-methyl]MSMA.

Characterization of Blood Residues

Attempts were made to characterize the test material residues present in whole blood of the composited 200 mg/kg dose group. Extraction efficiencies for male and female blood samples were 55.5 and 23.6%, respectively.

The blood extract added to the HPLC column could not be eluted from the column. The nature of the bound residue remains unidentified.

CONCLUSIONS:

Sprague-Dawley CD rats were treated by oral gavage with a single dose of [^{14}C-methyl]MSMA at doses of 0 (control), 5 or 200 mg/kg, 5 mg/kg MSMA orally for 14 consecutive days followed by a single oral dose of 5 mg/kg of [^{14}C-methyl]MSMA or a single i.v. injection of 5 mg/kg of [^{14}C-methyl]MSMA.

[^{14}C-methyl]MSMA was excreted in the urine and feces 24-48 hours after treatment, primarily as unchanged parent. The mean total radiocarbon recovered as $^{14}CO_2$ accounted for ≤0.5% of the administered dose in all treated groups.

During the 7 days following dosing, the mean total recovery of radiocarbon in urine and feces was 91.3% for males and 88.8% for females. Specifically, the oral dose group (both sexes) excreted a mean of 41% of the administered dose in the urine and 48% in the feces; the i.v. group excreted 51% of the radiocarbon in the urine. Comparison of the radiocarbon excreted in the urine by the i.v. dosed rats with that excreted by the orally dosed rats indicated that approximately half of the oral dose was absorbed by the low and consecutive dosed animals and somewhat less by the high dosed animals.

A mean of 10, 6, 13 and 3% of the administered dose remained, respectively, in the tissues and carcass at the 5 mg/kg, 200 mg/kg, consecutive and i.v. infusion dose groups. In all groups, the largest fraction of the bound radiocarbon was accounted for in the blood. The other organs contained lesser amounts [e.g. up to 0.72% (liver), 0.24% (kidney) or 0.38% (spleen)].

Total mean recovery of radiocarbon in all treated groups was 98.2% of the administered dose. It is unclear, whether the residual bound radioactivity in tissues results from residual blood-bound radioactivity or from actual tissue-bound radioactivity.

014343

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EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: ONCOGENICITY-MOUSE
[OPPTS 870.4200 (§83-2b)]
MRID NO. 42173201

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Robert H. Ross

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: for D. L. Fefee
Date: MAY 22 2000

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T.

Signature: HT Borges
Date: MAY 22 2000

Robert H. Ross

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 22 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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EXECUTIVE SUMMARY

MONOSODIUM METHANEARSONIC ACID - (MSMA)

STUDY TYPE: ONCOGENICITY-MOUSE
[OPPTS 870.4200 (§83-2b)]
MRID NO. 42173201

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-33

Primary Reviewer:
Donna L. Fefee, D.V.M. Signature:_____
Date: _____

Secondary Reviewers:
H.T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

Supplement to Tox Document 009382, review for Accession 42173201, 104-week oncogenicity study-mouse. This supplement provides an executive summary to update the original review.

EPA Reviewer: A. Lowit, Ph.D. Date: 8/28/00
Reregistration Branch 2 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 8/31/00
Reregistration Branch 4 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 104-Week Oncogenicity - Mouse [OPPTS 870.4200 (§83-2b)]

DP BARCODE: D265953 SUBMISSION CODE: S579557
PC CODE: 013803 TOX CHEM NO: 582

TEST MATERIAL: Methanearsonic Acid (purity 98.7-99.8%)

SYNONYM: Monosodium acid methanearsonate, MSMA

CITATION: Gur, E., M. Pirak, T. Waner (1991) Methanearsonic acid - oncogenicity study in the mouse. Life Science Research Israel, Ltd., P.O. Box 139, Ness Ziona 70 451 Israel. Laboratory Report No. LSRI Project Number PAL/023/MAA, July 8, 1991. MRID 42173201. Unpublished.

SPONSOR: Luxembourg Pamol, Inc., 5100 Poplar Ave., Suite 2746, Memphis, TN 38137.

EXECUTIVE SUMMARY: In an oncogenicity study (MRID 42173201), methanearsonic acid (purity 98.7-99.8%; Batch No. 107/84) was administered in the diet to 52 Charles River $C_3B_6F_1$ mice/sex/dose at dose levels of 0, 10, 50, 200, and 400 ppm (0, 1.8, 9.3, 38, and 83 mg/kg/day for males and 0, 2.2, 12, 46, and 104 mg/kg/day for females) for 104 weeks.

There was no treatment related effect on mortality. Treatment related clinical signs of loose and mucoid feces in both sexes at 400 ppm were observed beginning at week 40 and continued until study termination. Females of the 200 and 400 ppm groups exhibited increased incidences of hypersensitivity (2/52, 8/52 and 11/51 for control, 200, and 400 ppm, respectively) and tonic convulsions (1/52, 9/52 and 12/51 for control, 200, and 400 ppm, respectively).

Mean absolute body weights were decreased in both sexes at 400 ppm from week 51 through termination (males: 83-86% of controls; $p<0.001$, females: 78-83% of controls; $p<0.001$), and overall weight gains for weeks 0-104 were decreased at the 400 ppm dose level for males (35% less than controls) and at the 200 and 400 ppm dose levels for females (18 and 46% less than controls, respectively). Food consumption by the females of the 400 ppm group was increased from week 47 until termination (15.8% greater than controls). Mean water consumption was increased in males of the 200 ppm group at weeks 51 and 75 (107-126% of controls; $p<0.05$ or $p<0.001$) and in males of the 400 ppm group from week 45 through termination (143-169% of

controls; $p<0.001$). Mean water consumption was increased in females of the 200 ppm group from week 45 through termination (116-135% of controls; $p<0.001$) and in females of the 400 ppm group from weeks 25 through termination (110-179% of controls; $p<0.01$ or $p<0.001$).

No remarkable hematological findings were observed. Clinical chemistry was not observed in this study. Spleen weights adjusted for body weight in females of the 200 and 400 ppm groups were statistically decreased as compared with controls; however, no corresponding gross or microscopic changes were noted.

Increased incidences of mucoid, foamy, fluid or soft cecal contents were noted in males at the 400 ppm dose level (4/51 vs. 0/52 for controls) and in females at the 200 and 400 ppm dose levels (2/52, 4/52, and 12/52 for 0, 200, and 400 ppm females, respectively). The histopathology finding of diffuse, slight cuboidal to squamous metaplasia of the surface epithelial columnar absorptive cells of the cecum, colon, and rectum was observed at increased incidences ($p<0.001$) in males and females at 400 ppm (range of incidence 14/52 to 39/49; none observed in control). The finding of slight, subchronic progressive glomerulonephropathy exhibited a positive significant trend ($p<0.001$) in males (25/52, 27/52, 38/52, 39/52, and 46/52 for control 10, 50, 200, and 400 ppm, respectively). The finding of slight, focal nephrocalcinosis exhibited a positive significant trend in males ($p<0.001$; 25/52, 30/52, 30/52, 45/52, and 45/52 for control 10, 50, 200, and 400 ppm, respectively) and females ($p<0.01$; 0/52, 1/52, 1/52, 2/52, and 5/52 for control 10, 50, 200, and 400 ppm, respectively).

Based on decrease in body weight gain, increased water consumption, and histopathology of the large intestine and kidney, the LOAEL was 200 ppm (38 and 46 mg/kg/day) for males and females. The NOAEL was 50 ppm (9.3 and 12 mg/kg/day) for males and females.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate.

This study is classified **Acceptable/Guideline** and satisfies the guideline requirements for a combined chronic toxicity/oncogenicity study in rats [OPPTS 870.4200 (§83-2b)].

Attachment 1

The following attachment is not available electronically
See the file copy.

014343

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

009382

MAR 25 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

Subject: Review of Toxicology Studies with Methanearsonic Acid to support reregistration of the test substance. (Toxchem Number 582, HED Project No. 2-1208; Barcode number: D173830)

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 3/15/92
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Barbara Briscoe PM (51)/Betty Crompton PM Team Reviewer
Special Review and Reregistration Division
HED (H7508W)

THRU: Elizabeth Doyle, Ph.D., Section Head E.A. Doyle 3/17/92
Tox. Section II, Review Section IV
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and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 3/18/92
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ACTION REQUESTED: Review of toxicology studies for reregistration requirements.

Study Summarized

MRID 421732-01, Oncogenicity Study - mouse (83-2); Core - guideline.

Methanearsonic acid was incorporated into the diet of 5 groups of 52 mice/sex/group at concentrations of 0, 1.3, 9.3, 38 and 83 mg/kg/day (males) and 0, 2.2, 12, 46 and 104 mg/kg/day (females) for 104 weeks.

No evidence of carcinogenicity was seen.

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Mortality was not affected by treatment. In the high and high intermediate dose animals of both sexes, signs of toxicity occurred after 10-12 weeks of treatment. Loose and mucoid feces were seen at the high dose. A decrease in the mean body weight gain and an increase water intake occurred in the high dose of both sexes and the high-intermediate dose females. Food consumption was increased in the high dose females.

The colon, cecum and rectum showed a slight degree of diffuse cuboidal and squamous metaplasia in both sexes at the high dose versus the control.

The MTD (Maximum Tolerated Dose) = 83 mg/kg/day (Highest Dose Tested) - males; 46 mg/kg/day - females.

NOEL (No observed effect level) = 38 mg/kg/day (males); 12 mg/kg/day (females).

LOEL (Low observed effect level) for systemic toxicity = 83 mg/kg/day in males; 46 mg/kg/day in females.

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Reviewed by Steven L. Malish, Ph.D. Steven L. Malish 3/17/92
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E. A. Doyle 3/17/92
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE: Oncogenicity Study (83-2) - Mouse

MRID NO: 421732-01

TEST MATERIAL: Methanearsonic Acid

SYNONYMS: MAA

SPONSOR: Luxembourg Pamol, Inc.
5100 Poplar Ave.
Suite 2746
Memphis, TN 38137

TESTING FACILITY: Life Science Research Israel. Ltd.
PO Box 139,
Ness Ziona, 70 451 Israel

LAB STUDY NO.: LSRI Project Number PAL/023/MAA

TITLE OF REPORT: Methanearsonic Acid
Oncogenicity Study in the Mouse

AUTHORS: E. Gur, M. Pirak, T. Waner

REPORT ISSUED: July 8, 1991

CONCLUSIONS:

Methanearsonic acid was incorporated into the diet of 5 groups of 52 Charles River C3B6F1 mice per sex at concentrations of 0 (Control), 10, 50, 200 and 400 ppm for 104 weeks.

Mortality was not affected by treatment.

In the high and high-intermediate dose of both sexes, signs of toxicity were seen after 10-12 months of treatment. Loose and mucoid feces were seen at the high dose. A decrease in the mean body weight gain and an increase in water consumption occurred in the high dose of both sexes and the high-intermediate dose females. Food consumption was increased in the high dose females.

The colon, cecum and rectum showed a slight degree of diffuse cuboidal and squamous metaplasia in both sexes at the high dose versus the control.

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Under conditions of this study, methanearsonic acid showed no evidance of carcinogenicity.

The Maximum Tolerated Dose (MTD) based on a decrease in the mean body weight gain = 400 ppm (82.8 mg/kg/day, HDT) - males; 200 ppm (46.4 mg/kg/day) - females.

NOEL = 200 ppm (37.5 mg/kg/day) - males; 50 ppm (11.5 mg/kg/day) - females.

LOEL (systemic toxicity) = 400 ppm (82.8 mg/kg/day) - males; 200 ppm (46.4 mg/kg/day) - females.

CLASSIFICATION: Core: guideline

The study satisfies the guideline requirement (83-2) for an oncogenicity study.

QUALITY ASSURANCE:

The final document had a signed quality assurance statement attesting to the fact that GLP guidelines were followed during the course of this study.

FLAGGING CRITERIA:

The final document had a signed statement noting that the EPA flagging criteria (40CFR 158.34) for potential adverse effects was applied and concluded that "this study neither meets nor exceeds any of the applicable criteria". The reviewer agrees with the final document's conclusion.

A. MATERIALS:

1. Test Compound

Chemical:	methanearsonic acid
Trade Name:	MAA
Label:	1) "Pamol Arad Ltd - Luxembourg Chemicals", (Consignment 1) 2) MAA 1 kg % AI 99.8% w/w; batch 107/84, 1/8/89 (Consignment 2).
Batch No.	107/84 in 2 consignments
Purity:	98.7 - 99.8% (Lab Analysis)
Description:	white crystals
Storage:	room temperature

a. Analyses of Formulated Diets:

The stability and homogeneity results noted below prove that the mixing technique produced a homogenous and stable mixture.

Stability

The week 1 diets were analyzed for stability on day 0 (all doses) and day 12 (low and high dose). The week 8 (low and high doses) diets were analyzed 12 days after preparation. The test material was within -7.5 to 9.5% of the required concentration during the 12 day periods.

Homogeneity

Homogeneity of MAA dispersal in the rodent diet was initially determined from the diet mix prepared on Weeks 1 and 8 of the study. The mixture was sampled from 6 different spots in the mixing vessel from each concentration and analyzed for MAA.

The percentage change from the theoretical value ranged from -13% at 10 ppm to -9% at 400 ppm.

Content Check

Checks were made to verify the test material content 11 times during the first 13 weeks and every 2 weeks, thereafter, until week 107. Samples were taken at each concentration level. The 10 ppm sample ranged from 6.0 to 11.2 ppm, the 50 ppm from 35 to 58 ppm, the 200 ppm from 155 to 236 ppm and the 400 ppm from 343 to 438 ppm.

2. Test Animals

Species: Mice
Strain: Charles River C3B6F1
Age: 3 weeks of age upon receipt
Weight: Males 6.2 - 16.6 gm; females 8.7 - 16.1 gm on arrival (10% of the sample weighed)
Source: Charles River Breeding Laboratories, Wilmington MA., USA.

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B. STUDY DESIGN:

1. Animal Assignments

Animals were allocated to cages by a table of random numbers and housed singly. On commencement, all animals were weighed and any animal exceeding the mean value of the sex by 20% was replaced.

Fifty-two (52) animals per sex were assigned randomly to five (5) test groups and administered 0 ppm (group 1), 10 ppm (Group 2) 50 ppm (group 3), 200 ppm (Group 4) or 400 ppm (group 5) of the test material ad-mixed in the feed (Table 1).

Table 1

Animal Test Group Assignments[1]

Group	Treatment	Dietary Level[2] (ppm)	Animals on Test (M/F)
1	Control	0	52/52
2	MAA	10	52/52
3	MAA	50	52/52
4	MAA	200	52/52
5	MAA	400	51[3]/53

[1]Adapted from original report Vol I, p. 20.
[2]The dietary concentrations were expressed in terms of the material as supplied.
[3]After commencement of the study, animal No. 250 was found to be a female and discarded from the study.

2. Diet

Animals received the basal diet of Altromin 1321N chow (Altromin International Ltd., Lage, West Germany) and water ad libitum.

3. Diet preparation

Methanearsonic acid was incorporated into the powdered basal diet at the appropriate levels for the test diets each week. An initial premix was followed by dilution with further quantities of the diet and mixed. The dietary concentrations were expressed in terms of the material as supplied.

4. Water Supply

Drinking water was supplied to the cages via polyethylene bottles and stainless steel sipper-tubes. Water was taken from a water sterilizer connected to the public water supply and was routinely tested for physical, chemical and bacteriological characteristics.

5. Statistics

The significance of any intergroup differences in body weight performance, food consumption, water intake, absolute organ weight and hematology data was assessed as follows: homogeneity of variances was tested by the Bartlett's test. Where variances were homogeneous ($p>0.01$) then a parametric analysis of variance (ANOVA) was applied. When the F valuss were significant, Dunnett's multiple range test was applied for differences between control and treated groups. Where the variances were non-homogeneous the data was analyzed by the Kruskal-Wallis non-parametric ANOVA. If a significant difference between the groups was detected then the Dunn's test was applied for locating differences between the treated and control groups.

Survival function estimates were analyzed by the SAS Lifetest™ Procedure censoring for accidental deaths and scheduled sacrifice.

Organ weights were also analyzed using necropsy body weight as a covariant. Where a significant differences was found between the groups and this was at least partially accountable for by treatment ($p<0.05$), the multiple "t-tests" were applied for locating differences between the treated and controls.

Methods used in testing the significance of intergroup differences in pathology findings were described in Volume VI of the original report.

C. METHODS AND RESULTS:

1. Observations

Animals were observed daily for signs of ill health or toxic reaction to treatment. Animals were examined at least once weekly. Palpable swellings were identified as to location and described as to appearance, consistency and size.

2. Mortality

Mice killed in moribund condition, surviving to terminal sacrifice or dying during the course of the study were subjected to a gross necropsy. Moribund mice and those surviving until the end of the treatment period were killed by carbon dioxide inhalation.

Mortality was not affected by treatment. At commencement of terminal sacrifice, the survival rate among the males, in group order (beginning with the controls) were 87%, 87%, 90%, 83% and 92% and in the females 79%, 85%, 85%, 85% and 83%.

3. Clinical Signs

All treatment related observations were apparent starting from Week 40 until terrination of the study.

Loose and mucoid feces were noted in both sexes of the high dose group. High and high-intermediate dose females showed an increased incidence of hypersensitivity to touch and tonic convulsions versus the lower doses and the controls. No change was noted in the male treated groups (Table 2).

Table 2

Principle Clinical Signs of Toxicity Throughout the Study[1]

	Group and Sex									
Observations[2,3]	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Feces loose	0	0	0	0	20	2	0	0	2	34
Feces mucoid	1	0	0	2	47	6	2	1	5	50
Hypersensitive	5	1	2	1	4	2	4	2	8	11
Tonic Convulsions	10	1	6	5	6	1	6	3	9	12

[1]Adapted from the original report Vol I p. 73 thru 86.
[2]Number of animals showing sign during the study.
[3]No statistical calculation performed.

4. Body Weight

Each animal was weighed on the first day of treatment, at weekly intervals for the first 13 weeks, bimonthly till Week 99 and weekly until termination of the study.

The mean body weight gain in high dose males was reduced by -17% when averaged throughout the study (Table 3). A decrease in the absolute body weight of the high dose males, as evidenced by a statistical difference from the control, was seen starting about week 45 and continued until termination.

In females, a reduction in the mean body weight gain of -18% and -46%, was noted in the high-intermediate and high dose levels, respectively, when averaged throughout the study (Table 3). A decrease in the absolute body weight of the high dose females, as evidenced by a statistical difference from the control, was seen starting about week 43 and continued until termination.

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Table 3

Mean Body Weight (gm) at Selected Intervals Throughout the Two Year Study[1]

	Group and Sex									
Week	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
0	21	21	21	22	21	18	19	18	18	19
5	25	25	24	25	24	21	21	21	21	21
25	32	32	32	32	31	27	27	28	28	27
51	37	38	38	38	32[c]	35	35	34	33	29[c]
75	40	42	41	41	34[c]	38	38	38	36	30[c]
104	41	43	42	41	34[c]	40	38	38	36	31[c]
Change[d]	20	22	21	19	13	22	20	20	18	12
%[e]	--	10	5	-5	-35	--	-9	-9	-18	-46

[1]Adapted from original report, Vol I, p. 87 thru 93.
[c]significantly different from control, $p<0.001$.
[d]Change in weight from 0 week values. No statistical calculations performed.
[e]Percent difference compared to the control.

5. Food Consumption and Food Conversion Ratio

Food consumption was measured weekly for the first 13 weeks of treatment and biweekly, thereafter. The mean group intake was calculated at each time period.

In the female high dose level, food consumption increased 15.8% versus the control from week 47 until termination. Food consumption at the lower levels and in the males at all levels were considered to be not remarkable.

The food conversion ratio was considered to be not remarkable in both sexes throughout the course of the study.

6. Compound Consumption

Compound consumption expressed as mg/kg/day was calculated for each group/sex (Table 4).

Table 4

Mean Compound Consumption for Weeks 1-104[a,b,c]

Group	Males (mg/kg/day)	Females (mg/kg/day)
1	0.0	0.0
2	1.8	2.2
3	9.3	11.5
4	37.5	46.4
5	82.8	103.5

[a]Adapted from original report, Vol I, p. 101 thru 102.
[b]Calculated from the average food consumption and body weight at all time intervals during the study.
[c]Mean compound consumption calculated by the reviewer.

7. Water Intake

Water intake was measured weekly for the first 13 weeks of treatment and biweekly, thereafter, for all groups.

In the male high dosage group, water intake from week 45 until termination of the study was significantly increased ($p<0.001$). A similar pattern of water intake was apparent in the females. From week 41, an increased water intake was apparent in females of both the high and high-intermediate dose groups ($p<0.001$) until termination of the study. Compared to the respective controls, the high dose was 33% and 42% higher in the males and females, respectively and 17% higher in the high-intermediate females (Table 5).

Table 5

Mean Water Consumption (ml/animal/week) at Selected Intervals Throughout the Two Year Study[1]

	Group and Sex									
Week	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
1	47	44	43[a]	43[a]	44	46	45	45	46	48
5	45	46	44	43	47	46	48	46	48	49
25	40	38	38[a]	38	39	39	42[a]	40	41	43[b]
45	37	36	36	39	53[c]	38	40	39	46[c]	61[c]
51	35	36	37	44[c]	59[c]	40	41	42	54[c]	64[c]
75	44	42	45	47[a]	71[c]	42	43	43	53[c]	75[c]
103	42	40	38	44	65[c]	45	47	46	52[c]	72[c]
Mean[d]	40	39	39	42	53	41	43	43	48	58
%[e]	--	-3	-3	5	33	--	5	5	17	42

[1] Adapted from original report, Vol I, p. 104 thru 110.
[a] significantly different from control, $p<0.05$.
[b] significantly different from control, $p<0.01$.
[c] significantly different from control, $p<0.001$.
[d] Mean of observations throughout study. No statistical calculations performed.
[e] Percent difference compared to the control.

8. Clinical Pathology

Blood was collected from the tail after 12, 18 and 23 months for differential white blood cell counts from groups 1 and 5 only. The following cell types were scored: neutrophils, lymphocytes, eosinophils, monocytes and normocytes.

The differential counts were not remarkable throughout the course of the study.

9. Sacrifice and Pathology

Animals in extremis and those that completed their scheduled test period were sacrificed by carbon dioxide inhalation.

The study was terminated after 104 weeks of treatment. Terminal sacrifice was undertaken during weeks 105-108. Animals continued to receive the treated diet until necropsy.

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All animals that died or were scheduled for sacrifice were subject to gross and pathological examination. The checked (X) tissues were collected for gross and histological examinations from the control (Group 1) and high (group 5) dose groups. Organs and tissues denoted by an (^^) were examined in Groups 2, 3 and 4. The (XX) organs were weighed. Organs and tissues marked with a (*) were required by the 83-2 guidelines.

Organs and Tissues Examined Histopathologically at the Terminal Sacrifice

Digestive	Cardiovas./ Hematology	Neurologic
	X aorta*	XX brain*
X esophagus*	XX heart*	
X stomach*^^(d)	X bone marrow*(e)	X spinal cord (3 levels)
X duodenum*^^	X lymph nodes*	X sciatic nerve^^(c)
X jejunum*	cervical/mesen	X pituitary*
	/abdominal	X eyes* & optic nerve*
X ileum*	XX spleen*(c)	Glandular
X cecum*^^	X thymus*	X adrenals*^^
X colon*^^	Urogenital	X parathyroids*^^
X rectum*^^	XX kidney*^^	X thyroids*^^
XX liver*^^	X urinary bladder	Other
X pancreas*	XX testes*(a)	X bone*(e)
Respiratory	X prostate*	X skeletal muscle*
X trachea*	X seminal ves.	X skin*
XX lung*^^	X ovaries*	X gall bladder*
	X uterus*(b)	X Harderian gland
		X salivary gland*
		X abnormalities^^*
		X skull (nasal passages)

X Groups 1 and 5 examined microscopically
XX weighed and examined microscopically.
^^ microscopic examination from Groups 2, 3 and 4.
* specified by the guidelines
(a) with L and R epididymides and seminal vesicles
(b) corpus and cervix, (c) males only, (d) fundus, pylorus
(e) sternum including marrow, tibia femoral joint

a. Organ Weights

Organ weights were excluded from calculations where the organ had a visible mass or other abnormality at necropsy. Similarly, outlying values were also excluded.

No significant differences in the absolute organ weights were apparent at necropsy.

When the spleen was analyzed using the body weight as the covariant, differences were noted in the spleen and liver. The spleen in the female high and high-intermediate doses were lighter than the controls. The difference was mainly attributed to treatment (Table 6).

Table 6

Spleen Weight Using Body Weight As A Covariant
Least Square Means[1]

Group/ Males	LSMean[2] (gm)	Group/ Females	LSMean[2] (gm)
1	0.10	1	0.22
2	0.09	2	0.18
3	0.09	3	0.21[b]
4	0.08	4	0.15[b]
5	0.07	5	0.15[a]

[1]Adapted from original report, Vol I, p. 116-117.
[2]LS = least square mean (measured in gm)
[a]significantly different from control, $p<0.05$.
[b]significantly different from control, $p<0.01$.

b. Pathology Findings

1. Gross Pathology Findings:

A slight increased incidence of abnormal cecal contents was noted among high dose level animals (Table 7).

Table 7

Incidence of Abnormal Cecal Contents Related to Treatment with Methanearsonic Acid[a,b]

	Group and Sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Animals Examined	52	52	52	52	51	52	52	52	52	52
CECUM contents:										
mucoid, foamy, fluid or soft	0	0	0	0	4	2	0	1	4	12

[a]Adapted from the original report p. 36.
[b]No statistical evaluation performed.

2. Microscopic Pathology

Digestive Tract

Changes considered related to treatment were noted in the rectum, colon and cecum. Other lesions possibly related to treatment were seen in the kidney and adrenal glands.

The large intestine of both male and female high dosage animals showed a lesion consisting of diffuse, slight cuboidal to squamous metaplasia of the surface epithelial columnar absorptive cells (Table 8).

Table 8

Lesions of the Digestive Tract Related to Treatment with Methanearsonic Acid[1]

	Group and Sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
CECUM - No. examined	49	52	49	50	49	47	50	48	52	52
Epithelial columnar absorptive cells to cuboidal to squamous metaplasia: D,S.	0	0	0	0	29[c]	0	0	0	0	35[c]
%[2]	-	0	0	0	59	-	0	0	0	67
COLON - No. examined	51	52	51	50	49	49	0	49	51	52
Epithelial columnar absorptive cells to cuboidal to squamous metaplasia: D,S.	0	0	0	0	14[c]	0	0	0	0	17[c]
%	-	0	0	0	29	-	0	0	0	33
RECTUM - No. examined	50	52	51	52	49	50	50	51	51	52
Epithelial columnar absorptive cells to cuboidal/squamous metaplasia: D,.S.	0	0	0	0	39[c]	0	0	0	0	42[c]
%	-	0	0	0	80	-	0	0	0	81

[1] Adapted from the original report p. 41.
[2] % = Lesion/number of animals.
[c] significantly different from control, $p<0.001$.
D = diffuse, S = slight

Kidney

Two changes, progressive glomerulonephropathy in males ($p<0.001$) and nephrocalcinosis in males ($p<0.001$) and females ($p<0.01$) showed a positive, significant trend which was considered as possibly related to treatment (Table 9).

Table 9

Lesions of the Kidney Possibly Related to Treatment with Methanearsonic Acid[1]

	Group and Sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
No. of kidneys exam.	52	52	52	52	51	52	52	52	52	52
Progressive glomerulonephropathy:										
slight, subchronic	25	27[c]	38[c]	39[c]	46[c]	7	5	10	3	3
moderate, subchronic	0	0	1	1	0	1	0	0	1	0
slight, chronic	0	0	0	0	0	1	0	0	0	0
%	48	52	75	77	90	17	10	19	8	6
Nephrocalcinosis:										
slight, focal	25	30[c]	30[c]	45[c]	45[c]	0	1[b]	1[b]	2[b]	5[b]
%	48	58	58	87	88	0	2	2	4	10

[1]Adapted from the original report p. 42.
[b]positive significant trend $p<0.01$.
[c]positive significant trend $p<0.001$.

DISCUSSION:

No treatment related mortality occurred.

Signs of toxicity started at approximately 10-12 months after commencement of treatment in the high dose males and the high and high-intermediate dose females as evidenced by clinical signs, decreased rate of weight gain, increased water and decreased food consumption as noted below.

Clinical signs observed in both sexes at the high dose level versus the respective controls were loose and mucoid feces while high and high-intermediate dose females showed an increased incidence of hypersensitivity to touch and tonic convulsions versus the lower doses and the controls (Tables 2).

A decrease in the mean body weight gain occurred throughout the study in both sexes when compared to the controls. In the high dose males, a decrease of -35% was noted while in the high-intermediate

and high dose females a reduction of -18% and -46% was seen, respectively. A decrease in the absolute mean weight body weight occurred starting at week 45 in the high dose males and week 43 in the high dose females which continued until termination (Table 3). High dose level females also showed a decrease in food consumption of 16% starting on week 47 and continuing until termination.

After approximately 10-12 months of treatment, the mean water intake was also increased throughout the study in the females of the high-intermediate and high dose groups and males at the high dose level (Table 5).

The absolute org. . weight of the spleen versus the controls were not remarkable. Analysis using body weight as the covariant indicated a treatment related decrease in the spleen weight at the high and high-intermediate dose level. This finding was not supported by any macroscopic or microscopic effect and was not dose related. The biological significance of this finding was unknown (Table 6).

Pathology changes were observed in the rectum, colon and cecum of both sexes. The changes were considered to be slight and were characterized by conversion of the normal absorptive columnar epithelium to diffuse cuboidal to squamous metaplasia (Table 8).

Epithelial metaplasia is usually associated with chronic irritation or endocrine imbalance and can be presented as substitution of a columnar mucous-secreting surface by a stratified squamous epithelial surface. Metaplasia can usually be considered an adaptive response of an epithelium and its significance can range from a simple adaptive response to a pre-neoplastic change. No neoplastic changes related to treatment with the test substance were noted in the large intestine.

In the kidney, two changes, progressive glomerulonephropathy in males and nephrocalcinosis in males and females showed a positive significant trend. These lesions were consistent with the normal spectrum of spontaneous renal lesions encounter in aged B6C3F1 mice and no difference in the character and/or severity of lesions were noted between groups. These lesions, were considered to be possibly related to the administration of the test material (Table 9).

CONCLUSIONS:

Methanearsonic acid was incorporated into the diet of 5 groups of 52 Charles River C3B6F1 mice per sex at concentrations of 0 (Control), 10, 50, 200 and 400 ppm for 104 weeks.

Mortality was not affected by treatment.

In the high and high-intermediate dose of both sexes, signs of toxicity were seen after 10-12 months of treatment. Loose and

mucoid feces were seen at the high dose. A decrease in the mean body weight gain and an increase in water consumption occurred in the high dose of both sexes and the high-intermediate dose females. Food consumption was increased in the high dose females.

The colon, cecum and rectum showed a slight degree of diffuse cuboidal and squamous metaplasia in both sexes at the high dose versus the control.

Under conditions of this study, methanearsoric acid showed no evidence of carcinogenicity.

The Maximum Tolerated Dose (MTD) based on a decrease in the mean body weight gain = 400 ppm (82.8 mg/kg/day, HDT) - males; 200 ppm (46.4 mg/kg/day) - females.

NOEL = 200 ppm (37.5 mg/kg/day) - males; 50 ppm (11.5 mg/kg/day) - females.

LOEL (systemic toxicity) = 400 ppm (82.8 mg/kg/day) - males; 200 ppm (46.4 mg/kg/day) - females.

Page_____ is not included in this copy.

Pages 227 through 231 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

DATA EVALUATION REPORT 014343

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METHANEARSONIC ACID

STUDY TYPE: MULTIGENERATION REPRODUCTION FEEDING - RAT
[OPPTS 870.3800 (§83-4)]

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-36

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K.A. Davidson, Ph.D., D.A.B.T.

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Signature: Robert H. Ross
Date: JUN 14 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JUN 14 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

DATA EVALUATION REPORT

METHANEARSONIC ACID

STUDY TYPE: MULTIGENERATION REPRODUCTION FEEDING - RAT
[OPPTS 870.3800 (§83-4)]
MRID 43178301.

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 00-36

Primary Reviewer:
K.A. Davidson, Ph.D., D.A.B.T. Signature:
Date:

Secondary Reviewers:
Carol Forsyth, Ph.D. Signature:
Date:

Robert H. Ross, M.S. Group Leader Signature:
Date:

Quality Assurance:
Lee Ann Wilson, M.A. Signature:
Date:

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: A. Lowit, Ph.D. _____, Date 7/17/00
Reregistration Branch 2: (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. _____, Date 8/31/00

Reregistration Branch 4 (7509C)

014343

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - rat [OPPTS 870.3800 (§83-4)]

DP BARCODE:D265953 SUBMISSION CODE: S579557
P.C. CODE: 013803 TOX. CHEM. NO.: 582

TEST MATERIAL (PURITY): Methanearsonic acid (99.44% a.i.)

SYNONYMS: methylarsonic acid, methylarsinic acid, momomethylarsinic acid

CITATION: Schroeder, R. 1994. A two-generation reproduction study in rats with methanearsonic acid (MAA). Pharmaco LSR, Inc., East Millstone, NJ. Laboratory report number 91-3668, March 17, 1994. MRID 43178301. Unpublished

SPONSOR: MAA Research Task Force Three, c/o Dr. Elizabeth Owens, ISK Biotech, Inc., 5966 Heisley Rd., Mentor, Oh 44061

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 43178301), methanearsonic acid (MAA, 99.44% a.i., Batch No. 0030401) was administered to 30 F_0 and F_1 male and 30 F_0 and F_1 female CD® Sprague-Dawley derived rats per group at dietary concentrations of 0, 100, 300, or 1000 ppm. The dietary concentration corresponded to 5.6, 17.2, and 61.4 mg/kg/day, respectively, for F_0 and F_1 males averaged over the entire study and 7.5, 22.5, and 77.6 mg/kg/day, respectively, for F_0 and F_1 females averaged over the premating period. F_0 and F_1 males and females received treated or control food for a 14-week premating period; males remained on treatment until delivery of the last litter and females until weaning of the last litter. F_1 weanlings selected to produce the F_2 generation were weaned onto the same food as their parents.

Administration of MAA at doses of 100, 300, or 1000 caused no treatment-related effects on mortality or clinical signs in either F_0 or F_1 parental animals. Food consumption was in increased in F_0 and F_1 males of the 300 and 1000 ppm groups, F_0 females of the 1000 ppm group, and F_1 females of the 300 and 1000 ppm groups. Although increases in food consumption were increased, body weight and body weight gain were reduced by approximately 10% relative to control in males of the F_0 generation at 300 and 1000 ppm level and in males of the F_1 generation at 1000 ppm. These results of increased food consumption and decreased body weight gain are consistent with results from chronic feeding studies in mice (MRID no. 42173201) and rats (MRID no. 41669001) and are therefore considered treatment related. No effects on absolute body weights or body weight gain were observed in F_0 or F_1 females during the premating,

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gestation, or lactation periods. Food consumption was increased ($p > 0.05$) for female parental rats at 1000 ppm during the premating and gestation periods for both generations.

Absolute mean weights of the right and left testes (weighed separately) of 1000-ppm group F_0 males were 8% ($p<0.01$) less than that of controls. It is notable that the relative testes weights were only 3% less than control. In 1000-ppm F_1 group males, the absolute and relative prostate gland weighed 19% ($p<0.05$) and 13% less. Females in both generation administered the test material had organ weights similar to those of the controls (pituitary gland was the only organ measured in females).

In conclusion, the parental LOAEL was 300 ppm (17.2 mg/kg/day) for male rats and 1000 ppm (77.6 mg/kg/day) for female rats based on increased food consumption with decreased body weight gain. The parental NOAEL is 100 ppm (5.6 mg/kg/day) for males and 300 ppm (22.5 mg/kg/day) for females.

The evaluation of reproductive performance showed no treatment related effects on sperm/spermatid count, morphology, or motility. The mating index was decreased in F_0 males of the 300 and 1000 ppm groups due to fewer animals who actually mated successfully (24 vs 28 in control). The mating index was actually higher in males of the F_1 generation. The fertility index of F_1 females of the 300 ppm group in addition to F_0 and F_1 males and females of the 1000 ppm group was reduced relative to control due to a reduced number of pregnant females. It is noteworthy that although the fertility indexes for the groups noted above are within the range of historical controls included with the study (76.2-100.0 for males; 71.4-100% for females), the actual values are on the low-end of this historical range (74.1-79.2%). These equivocal effects on reproductive performance are corroborated by Prukop and Savage (1986) who performed a one-generation reproduction study in mice gavaged with MSMA at 11.9 or 119 mg/kg every other day for 10 weeks. Decreased male fertility was observed at both doses compared to control. Only 50% of the females at 11.9 mg/kg/dose and none of the females at 119 mg/kg/dose became pregnant.

The reproductive LOAEL is 300 ppm (17.2 mg/kg/day) based on decreased fertility indexes in both sexes. The reproductive NOAEL is 100 (5.6 mg/kg/day).

Decreased lactation index compared to concurrent and historical controls was observed for 300 ppm F_2 pups and 1000-ppm group F_1 and F_2 pups. The decrease in the lactation index is due primarily to a whole litter loss at both dose levels. There were no treatment related effects on any other pup data. Body weights and body weight gain of F_1 and F_2 pups were comparable to control values throughout lactation. The number of pups that died between day 0-21 was increased in 300 ppm F_2 pups (35) and 1000-ppm group F_1 and F_2 pups (35 and 32, respectively) compared to control (8 and 15, respectively). Because of pup death, the litter survival index was reduced in these noted groups.

This equivocal data on pup death is corroborated by the Prukop and Savage (1986) study. In two of four females in the 11.9 mg/kg/dose group that produced litters, the mothers "did not build a nest, rarely huddled over the young, or retrieved them when they were separated from the young, all of which are maternal instincts." In these two litters, all of the young mice died with 2 to 3 days of birth. This postnatal litter loss parallels postnatal litter loss observed in rats of the

guideline two-generation reproduction study. Additional behavioral affects were observed in the Lopez and Judd (1979) where females treated with MSMA produced significantly smaller nests than did controls. Based on this literature evidence, it is plausible that the whole litter loss observed in the two-generation reproduction study is due to changes in the nurturing behavior of the mothers and not a direct toxic effect on the pups. Therefore, the increased pup death is not an indication of infant susceptibility but rather potential neurotoxicity in the females.

The offspring LOAEL is 300 ppm (17.2 mg/kg/day) based on increased pup death (day 0-21), reduced litter survival index, and decreased lactation index. The offspring NOAEL is 100 (5.6 mg/kg/day).

The reproductive study in rats is classified **Acceptable/guideline** and satisfies the guideline requirement for a two-generation reproductive study [OPPTS 870.3800 (§83-4)] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, No Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methanearsonic acid (MAA)
   Description: white powder
   Lot/Batch #: 0030401
   Purity: 99.44% a.i.
   CAS #: 124-58-3

2. Vehicle
   Description: Purina Certified Rodent Chow® Bran Diet #5002 (meal)

3. Test animals

   Species: rat
   Strain: CD®Sprague-Dawley derived
   Age at start of dosing: F_0: 59 days
   Weight at start of dosing (F_0):
   males: 302.6±19.3 - 310.1±15.9 g; females: 195.4±16.4 - 196.2±14.6 g
   Source: Charles River Laboratories, Inc., Portage, MI
   Housing: Animals were housed individually in stainless steel, wire mesh bottom cages except as noted: acclimation (2/cage), mating (one male/one female), lactation (dam with litter), and post-weaning (2/cage until selection). Stainless steel floor pan and hardwood bedding were placed in each cage for mated females from gestation day 20 to lactation day 14.
   Diet: Purina Certified Rodent Chow® Bran Diet #5002 (meal), *ad libitum*
   Water: tap water, *ad libitum*
   Environmental conditions:
   Temperature: 66 - 77 F

Humidity: 28 - 75%
Air changes: not reported
Photoperiod: 12 hours light /12 hours dark
Acclimation period (F_0): 4 weeks

B. PROCEDURES AND STUDY DESIGN

1. In life dates

Start: May 20, 1992 end: March 29-31, 1993 (males); April 15-16, 1993 (females)

2. Mating procedure

One male and one female from the same treatment group were caged together nightly for 7 consecutive days or until evidence of mating was observed as judged by sperm in the vaginal smear or a copulation plug was found. If no evidence of mating was observed after 7 days, the female was caged with another randomly selected male from the same treatment group for 7 days or until evidence of mating was observed. This procedure was repeated up to three times for unmated females. The report did not mention sibling matings; the reviewer assumes that sibling matings were avoided.

After successful mating or three trials, each female was individually placed in a cage with a stainless-steel floor pan and hardwood bedding through day 14 of lactation.

3. Study schedule

The test or control (basal) diet was administered continuously to each group of F_0 female rats for 14 weeks prior to mating, throughout mating, gestation, and lactation of the F_1 litters. F_0 males were administered the same diets as the females continuously from study initiation until sacrifice following delivery of the last F_1 litter. F_0 males and females were about 157 days old at the time of mating. Two F_1 pups of each sex per group were selected at weaning to form a pool from which one of each sex per group was culled after all litters were weaned leaving the desired number of F_1 weanlings to parent the next generation. The F_1 female pups selected to parent the next generation were administered the same diets as their parents during the post-weaning period and for 14 weeks prior to mating, throughout mating, gestation, and lactation of the F_2 litters. F_1 males selected to produce the next generation received the same diets as their parents from the time of weaning until sacrifice following delivery of the last F_2 litter.

4. Animal assignment

F_0 animals were randomly assigned to test groups listed in Table 1 based on body weights; the heaviest and lightest animals were excluded from the study.

TABLE 1. Animal assignment

Test Group	Conc. in Diet[a] (ppm)	Animals/group

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		F_0		F_1	
		Males	Females	Males	Females
Control	0	30	30	30	30
Low (LDT)	100	30	30	30	30
Mid (MDT)	300	30	30	30	30
High (HDT)	1000	30	30	30	30

Data were taken from page 18, MRID 43178301.
[a]Diets were administered from the beginning of the study until delivery of last litter (males) or weaning of last litter (females).

5. Dose selection rationale

The doses were selected by the sponsor. The dose selection rationale was based on a range-finding reproductive study; the results were not reported by the study author.

6. Dosage preparation and analysis

Test diets were prepared at 3-week intervals. An appropriate amount of test substance for each dose was ground to a uniform fine powder using a mortar and pestle. The powdered test material and untreated food used for transfer and rinsing were mixed in a Hobart mixer with a premeasured amount of untreated food for 10 minutes to prepare a premix. The premix and an appropriate amount of untreated food were mixed in a PK-Twinshell Mixer for 20 minutes. Each dietary formula was mixed separately. In a range-finding study, stability of the test substance in the diet was evaluated on samples of 200- and 1000-ppm dietary formulations stored for 14 days at ambient temperature and on 100- and 1000-ppm samples stored at ambient temperature for 21 days. Prior to initiation of the study, homogeneity was evaluated on full-size batches of the 100- and 1000-ppm diet; triplicate samples were taken from the top, middle, and bottom. During the study, samples of treated food from the first five mixes and every fourth mix (weeks 16, 22, 24, 26, 34, 42, and 47) were analyzed for verification of dietary concentration.

Results -
Homogeneity Analysis: 100 ppm: The mean of the triplicate samples taken from the three levels (top, middle, and bottom) ranged from 84% to 134% of nominal. **1000 ppm**: The mean of the triplicate samples ranged from 104% to 125% of nominal; the mean values upon re-analysis of these samples was 101.2% to 109.0% nominal (pg. 412).

Stability Analysis: 100 ppm: After 21 days of storage, the measured mean concentration was 110% of nominal (range 92-133%). **1000-ppm**: The sample concentration ranged from 88.5% to 108.7% of nominal after storage for 21 days. The study author did not report the results as percent of day 0 dietary concentration.

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Concentration Analysis: The concentrations of test material in the dietary preparations were measured by gas chromatography (GC) and atomic absorption spectroscopy (AAS). MAA was analyzed by GC and arsenic by AAS, which was converted to equivalent

concentrations of MAA. Considerable variations were observed between duplicate samples indicating problems with analytical precision. The concentration of test material in almost all formulations exceeded the target by more than 10% when measured by GC. A larger number had concentrations within ±10% of the target when analyzed by AAS.

The mixing procedure and stability appeared to be adequate. The lack of analytical precision of the methods employed to measure the concentration of test material in the dietary preparations made it difficult to judge the adequacy of the variance between nominal and actual dosage to the study animals.

C. OBSERVATIONS

1. Parental animals

Observations and the schedule for those observations are summarized in Table 2. In addition, observations for the F_1 generation were initiated with the beginning of the formal premating period (week 24). Around the time of expected delivery, pregnant or presumed pregnant females were observed twice a day for parturition. Females that did not mate were weighed weekly during mating until sacrifice. Food consumption was not measured for either sex during the mating period or during the lactation period for females. Food consumption was calculated as g/kg body weight/day. Food efficiency was not calculated.

TABLE 2. Observation schedule for F_0 and F_1 parental animals

Type of Observation	No. animals per sex per group	Time of observation	Frequency of observation
Mortality, gross signs of toxicologic or pharmacologic effects	All	Throughout study	Twice a day
Detailed physical observations with palpation for masses	All	Throughout study	Study weeks -2, -1, 0 then weekly until sacrifice
Body weights			
Males	All	F_0 only: weeks -3 to 0 (pretest) to sacrifice; F_1: week 24 (start of premating) until sacrifice	Weekly
Females	All	F_0 only: weeks -3 to 0 (pretest) and premating period, F_1: premating period (week 24 to mating)	Weekly
	All	Gestation	Day 0, 4, 7, 14, 20
	All	Lactation	Day 0, 4, 7, 14, 21
Food consumption			
Males	All	F_0: weeks -2 to 0 (pretest) to sacrifice (premating and postmating periods); F_1: week 25 to sacrifice	Weekly
Females	All	F_0: weeks -2 to 0 and premating period; F_1: week 25 to mating	Weekly
	All	Gestation	Day 0-7, 7-14, and 14-20

	All	Lactation	No measurements

Data taken from pages 25-27, MRID 43178301.

2. Litter observations

According to the report, the following litter observations (X) were made (see Table 3). Litters were observed as soon as possible after delivery on day 0 of lactation to determine the number of live and dead pups and pup abnormalities. Thereafter, litters were observed twice daily through day 21 of lactation and the numbers of live and dead pups were recorded as indicated in Table 3.

TABLE 3. F_1/F_2 Litter observations						
Observation	Time of observation (lactation day)					
	Day 0	Day 4[a]	Day 4[b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Pup weight	X	X		X	X	X
External alterations	Twice daily					
Sex of each pup (M/F)	X	X	X	X	X	X

Data extracted from pages 27-28, MRID 43178301.
[a]Before standardization (culling)
[b]After standardization (culling)

On day 4 postpartum, litters were standardized to a maximum of eight pups/litter with equal distribution between the sexes where possible. Dead, stillborn, or culled pups were examined grossly for external and internal abnormalities, internal sex identification, unusual observations, and the presence or absence of milk in the stomach. These pups were preserved in fixative.

3. Postmortem observations

1) Parental animals: All surviving parental males were sacrificed after delivery of the last litter and all surviving females were sacrificed soon after weaning of the last litter. The animals were exsanguinated after inhaling carbon dioxide. All adult F_0 and F_1 animals were subjected to a gross examination that included a count of uterine implantation sites in females.

Sperm evaluations that included the following were performed on 10 F_0 and 10 F_1 generation males randomly selected at the time of necropsy: spermatid count (left testis), total cauda epididymal sperm count (left cauda epididymis), sperm morphology (left cauda epididymis), sperm motility (left vas deferens), and assessment of fluid, debris, and unexpected cell types (left cauda epididymis). The following tissues (X) from all F_0 and F_1 generation adults were examined by light microscopy and the (XX) tissues weighed . The cauda epididymis was weighed separately when used for sperm evaluations.

X	Ovaries	X	Epididymides
X	Uterus	XX	Prostate
X	Vagina/cervix	XX	Seminal vesicles
XX	Pituitary gland	XX	Testes
X	Gross lesions	X	coagulating gland

2) Offspring: F_1 pups not selected to parent the next generation were sacrificed by carbon dioxide asphyxiation on day 21 of lactation or soon after weaning, and F_2 pups were sacrificed on day 21 of lactation; all these pups were subjected to gross external and internal examinations. All F_1 and F_2 pups that were stillborn, found dead, or culled on day 4 were weighed and also subjected to gross external and internal examinations.

D. DATA ANALYSIS

1. Statistical analyses:

Body weights, body weight gain, food consumption, gestation length, number of pups, pup weights and viability, weaning indices, organ weights, and sperm assessments were evaluated with Bartlett's test for equality of variances. If the variances were equal, data were analyzed by one-way ANOVA using the F distribution (parametric procedure) to test for statistical significance, followed by Dunnett's test for pairwise comparisons. If the variances were unequal, data were analyzed by the Kruskal-Wallis test followed by Dunn's rank sum test for pairwise comparisons. Trend tests for data with equal variances utilized standard regression techniques with a test for trend and lack of fit. Trend tests for data with unequal variances utilized Jonckheere's test for monotonic trend. Statistical significance was indicated $p<0.01$ (two-sided) for Bartlett's test and $p<0.05$ or $p<0.01$ for other statistical tests.

Mating and male fertility indices, pregnancy rates, litter survival index, and mortality rates (incidence data) were evaluated with the Chi-square test for comparison between groups tested; the Fisher exact test with Bonferroni correction was used to compare treatment groups with control and the Armitage test was used for linear trend. Statistical significance was indicated by $p<0.05$.

2. Indices

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study (see pages 47 and 162, MRID 43178301):

$$\text{Male (female) mating index} = \frac{\text{No. males (females) with evidence of mating (plug, vaginal sperm, or pregnancy)}}{\text{Total No. paired}} \times 100$$

$$\text{Male fertility Index} = \frac{\text{No. males producing at least one confirmed pregnancy}}{\text{No. that mated}} \times 100$$

$$\text{Female pregnancy (fertility) index} = \frac{\text{No. females pregnant}}{\text{No. that mated}} \times 100$$

$$\text{Gestation Index} = \frac{\text{No. females delivering litters with at least one live pup}}{\text{No. females pregnant}} \times 100$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study (see pages 50 and 173, MRID 43178301):

$$\text{Viability index} = \frac{\text{Total No. live pups on day 4 (precull)}}{\text{Total No. live pups on day 0}} \times 100$$

$$\text{Weaning (lactation) index} = \frac{\text{Total No. live pups on day 21}}{\text{Total No. live pups on day 4 (post-cull)}} \times 100$$

$$\text{Litter survival index} = \frac{\text{No. litters with live pups on day 21 (weaning)}}{\text{No. litters with live pups at birth}} \times 100$$

3. Historical control data

Historical control data were provided for reproductive performance, gestation length, and litter and pup data.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

Except as noted below all F_0 and F_1 male and female rats survived to scheduled termination. One 100-ppm group female died on gestation day 21; a red vaginal discharge was observed on gestation day 20. Necropsy showed 12 dead term pups and 2 early resorptions. One accidental death occurred among the control males during the postmating period. One 1000 ppm F_1 female died during the early phase of the premating period. The cause of death was unknown, but was not considered treatment related.

No treatment-related clinical signs were observed at any time during the study in F_0 or F_1 male or female rats receiving any dose of the test material.

2. Body weight and food consumption

Selected mean body weight, body weight gain, and food consumption data are summarized in Tables 4a for the F_0 generation and 4b for the F_1 generation.

Body weights and weight gain of F_0 females receiving the test material were similar to those of the controls throughout the premating period. Mean body weights and weight gain in F_1 females administered the test material were similar to control values throughout the premating period except for a 5-8% ($p<0.01$) elevation in body weights by the 100-ppm group during week 24-28.

Mean body weights of 1000-ppm F_0 males were not significantly different from that of controls except for a 5% ($p<0.05$) decrease at week 16, and body weight gain was

9% (N.S.) less than that of control for the premating/postmating period (entire treatment period). Mean body weights of 300-ppm group F_0 males were not significantly different from that of controls for the first 13 weeks of treatment; statistically significant ($p<0.05$) decreases of 4-5% were observed for weeks 14 to 21 (termination) except for one week. The 300-ppm group F_0 males gained 9-10% less weight than controls over the entire treatment period ($p<0.05$ for premating period only). Food consumption expressed as g/kg body weight/day was increased by 5-13% ($p<0.01$) during the entire treatment period in 1000-ppm group males, and by 4-6% ($p<0.01$ or <0.05) at sporadic times during premating and during the last 3 weeks of the postmating period in 300-ppm group F_0 males. F_1 males administered the 1000-ppm diet weighed 7-9% ($p<0.01$) less and gained 9% less weight than controls during the premating/postmating period.

Both male and female rats administered the 300- and 1000-ppm diets consumed significantly more food than control. The 300- and 1000-ppm group F_1 males consumed 5-11% ($p<0.01$) and 14-20% ($p<0.01$) more, respectively, and the females consumed 5-3% ($p<0.01$) and 11-16% ($p<0.01$) more, respectively. Food consumption by 1000-ppm group F_0 females was increased by 9-13% ($p<0.01$ or <0.05) from weeks 10-14 of the premating period. It is important to note that F_0 females in the 1000-ppm group also consumed 7-8% ($p<0.01$) more food than controls before treatment was initiated.

Food efficiency was not calculated for this study.

TABLE 4a. Selected mean body weights, body weight gain, and food consumption of F_0 male and female rats during the premating/postmating period in rats fed methanearsonic acid

Observations/study week	Dietary concentration (ppm)			
	Control	100	300	1000
F_0 Generation Males - Premating/Postmating				
Mean body weight (g)				
Week 0	310.1	305.4	302.6	306.7
Week 7	472.1	460.0	456.8	461.8
Week 14	557.5	539.6	524.3	537.8
Week 21	594.0	581.1	560.6* (94)	565.9 (95)
Mean weight gain (g)				
Weeks 0-14	247.4	234.2	221.8* (90)	231.1 (93)
Week 0-21[b]	283.9	275.7	258.0 (91)	259.2 (91)
Mean food consumption (g/kg b.w./day)				
Week 1	82.9	83.3	82.2	81.7
Week 14	48.1	49.5	50.9* (106)	54.1** (112)
Week 21	47.4	47.3	49.5* (104)	54.8** (116)
F_0 Generation Females - Premating				
Mean body weight (g)				
Week 0	196.1	196.2	196.1	195.4
Week 7	258.9	266.1	265.1	265.7
Week 14	289.5	301.1	291.6	299.3
Mean weight gain (g)				
Week 0-14	93.4	105.0	95.6	103.8
Mean food consumption (g/animal/day)				
Week 1	93.0	95.0	96.0	96.0
Week 14	64.8	65.5	66.5	69.8** (108)

Data extracted from Table 3 (pp. 90-101), 4 (pp. 102-103), 6 (pp. 118-129), 8 (pp. 142-143), and 10 (pp. 146-147), MRID 43178301.

[a]Numbers in parentheses are percent of control values.

[b]Calculated by the reviewer.

*$p<0.05$, **$p<0.01$, statistically significant compared with controls.

TABLE 4b. Selected mean body weights, body weight gain, and food consumption of F_1 male and female rats during the premating/postmating period in rats fed methanearsonic acid

Observations/study week	Dietary concentration (ppm)			
	Control	100	300	1000
F_1 Generation Males - Premating/Postmating				
Mean body weight (g)				
Week 24	211.7	217.6	194.1	192.3** (91)[a]
Week 31	464.2	455.8	444.6	431.1** (93)
Week 38	557.1	542.0	538.3	513.6** (92)
Week 44	601.0	582.1	580.9	545.9** (91)
Mean weight gain (g)				
Weeks 24-38	345.4	324.5	344.2	321.3 (93)
Weeks 24-44[b]	389.3	364.5	386.8	353.6 (91)
Mean food consumption (g/kg b.w./day)				
Week 25	103.7	102.7	112.0** (108)	119.0** (115)
Week 38	48.0	48.2	49.9	55.8** (116)
Week 44	44.6	46.0	47.6** (107)	51.8** (116)
F_1 Generation Females - Premating				
Mean body weight (g)				
Week 24	149.3	161.4** (108)	146.5	146.6
Week 31	251.1	262.9	254.6	250.8
Week 38	290.0	301.3	293.2	290.3
Mean weight gain (g)				
Weeks 24-38	140.6	139.9	146.7	143.5
Mean food consumption (g/animal/day)				
Week 24	105.1	102.2	114.8* (109)	117.2** (112)
Week 38	63.0	64.0	66.5* (106)	71.9** (114)

Data extracted from Tables 3 (pp. 96-101), 4 (pp. 104-105), 6 (pp. 124-129), 8 (p. 143), and 10 (p. 147).
[a]Numbers in parentheses are percent of control values.
[b]Calculated by the reviewer.
*$p<0.05$, **$p<0.01$, statistically significant compared with controls.

F_0 and F_1 females administered all doses of the test material had mean body weights and body weight gain values similar to those of controls throughout gestation and lactation with the following exceptions. The 100- and 300-ppm group F_1 females weighed significantly more (6% and 10%) than the controls on day 21 of lactation, and 300-ppm group F_1 females gained significantly more weight (291%, $p<0.01$) than control during lactation during the entire lactation period. F_0 and F_1 females administered the 1000-ppm diet consumed significantly more food than controls throughout gestation (7%-13%). Food consumption was not measured during lactation.

3. Test Substance Intake

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Mean daily mg MAA/kg body weight, based on food consumption and body weights calculated for the intervals food consumption was measured is presented in Table 5. The

study author did not state whether nominal or analytical dietary MAA concentrations were utilized for the calculations.

TABLE 5. Test Substance Intake (mean mg/kg body weight/day) for F_0 and F_1 male and female rats

Treatment Period	Male			Female		
	100 ppm	300 ppm	1000 ppm	100 ppm	300 ppm	1000 ppm
F_0 Generation						
Premating period only	5.8	17.8	63.5	7.5	22.5	77.6
Premating/postmating[a]	5.6	17.2	61.4	–	–	–
F_1 Generation						
Premating period only	6.5	21.1	75.8	7.9	25.4	88.6
Premating/postmating[a]	6.2	19.9	71.7	–	–	–

Data extracted from page 38 and Table 7 (pp. 130-132 and 136-138) and 11 (pp. 148-149), MRID 43178301.
[a]Mean values calculated by the reviewer from premating and postmating test substance intake.

4. Reproductive function

a. Estrous cycle length and periodicity: Estrous cycles of female rats receiving the test material were not evaluated in this study.

b. Sperm measures: Testicular spermatid count, caudal epididymal sperm count, percent motile sperm, and sperm morphology in male F_0 and F_1 rats receiving all doses of the test material were similar to those of controls.

c. Sexual maturation (F_1): The time to sexual maturation was not determined in this study.

5. Reproductive performance

Results for the parental animals are summarized in Table 6a (F_0 generation) and 6b (F_1 generation). Fewer 300- and 1000-ppm group F_0 males mated with females than did controls resulting in a mating index lower than that of controls at both dose levels. Statistical significance in comparison with concurrent controls was not achieved, and the mating index for historical control was 88.2% with a range of 70.8-100.0%. The fertility index for 1000-ppm group F_0 males and females (79% and 74%, respectively) was slightly lower than that of the corresponding controls (86% and 86%, respectively).

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Significantly fewer 1000-ppm group F_1 males that mated with females actually impregnated a female resulting in a statistically significant decrease in the F_1 male fertility index (77.8%, $p<0.05$) compared with concurrent controls (100.0%). The male fertility index for historical controls was 91.1% with a range of 76.2-100.0%. The fertility index for 1000-ppm group F_1 females was also less than that of controls (75.9% vs 89.3% for controls); statistical significance was not achieved and the historical control value was 89.4% with a range of

71.4-100.0%. The mating and fertility indices at other doses and the gestation index for females of both generations were unaffected by treatment with the test material.

TABLE 6a. Reproductive performance for the F_0 generation

Observation	Dietary concentration (ppm)			
	Control	100	300	1000
MALES				
Number paired with females	30	30	30	30
No. that mated[a]	28	27	24	24
No. impregnating a female[b]	24	23	22	19
FEMALES				
No. paired with males	30	30	30	30
No. that mated[c]	29	29	29	27
No. pregnant[d]	25	24	24	20
No. litters with at least one live pup	24	23	23	19
Gestation length (days)	22.4	22.5	22.6	23.1
INDICES				
Mating index				
Males	93.3	90.0	80.0	80.0
Females	96.7	96.7	96.7	90.0
Fertility index				
Males	85.7	85.2	91.7	79.2
Females	86.2	82.8	82.8	74.1
Gestation index	96.0	95.8	95.8	95.0

Data extracted from page 47, Table 16 (p. 162) and 17 (163), MRID 43178301.

[a]Mating confirmed with at least one female (plug, sperm, and/or pregnancy).

[b]Mated males with at least one female showing evidence of pregnancy (parturition and/or uterine implantation scars.

[c]Females showing evidence of mating (plug, sperm, and/or pregnancy).

[d]Females showed evidence of pregnancy (parturition and/or uterine implantation scars)

TABLE 6b. Reproductive performance for the F_1 generation

Observation	Dietary concentration (ppm)			
	Control	100	300	1000
MALES				
Number paired with females	30	30	30	30
No. that mated[a]	25	26	21	27
No. impregnating a female[b]	25	23	19	21*
FEMALES				
No. paired with males	30	30	30	29
No. that mated[c]	28	28	29	29
No. pregnant[d]	25	25	23	22
No. litters with at least one live pup	25	23	22	22
Gestation length (days)	22.3	22.2	22.6	22.5
INDICES				
Mating index				
Males	83.3	86.7	70.0	90.0
Females	93.3	93.3	96.7	100.0
Fertility index				
Males	100.0	88.5	90.5	77.8*
Females	89.3	89.3	79.3	75.9
Gestation index	100.0	92.0	95.7	100

Data extracted from page 47 and Tables 16 (p. 162) and 17 (p. 167), MRID 43178301.

[a]Mating confirmed with at least one female (plug, sperm, and/or pregnancy).

[b]Mated males with at least one female showing evidence of pregnancy (parturition and/or uterine implantation scars.

[c]Females showing evidence of mating (plug, sperm, and/or pregnancy).

[d]Females showed evidence of pregnancy (parturition and/or uterine implantation scars)

*$p<0.05$, statistically significant compared with controls.

5. Parental postmortem results

a) Organ weights: Absolute mean weights of the right and left testes (weighed separately) of 1000-ppm group F_0 males were 8% ($p<0.01$) less than that of controls. It is notable that the relative testes weights were only 3% less than control. In 1000-ppm F_1 group males, the absolute and relative prostate gland weighed 19% ($p<0.05$) and 13% less. Females in both generation administered the test material had organ weights similar to those of the controls (pituitary gland was the only organ measured in females).

b) Pathology

1) Macroscopic examination: No treatment-related gross findings were observed for male or female rats of either generation.

2) Microscopic examination: No treatment-related microscopic findings were reported for male or female rats of either generation. Some common lesions consisted of degeneration/atrophy of the germinal epithleium of the testis and corpora amylacia/mineralized granules in the alveolar lumen of the prostate of F_0 and F_1 male rats and dilated uterine lumen, and squamous cell hyperplasia of the cervix and vagina of F_0 and F_1 females.

B. OFFSPRING

1. Viability and clinical signs

No treatment-related clinical signs were reported for F_1 or F_2 pups.

Litter and pup survival data are summarized in Tables 7a (F_1 generation) and 7b (F_2 generation). For F_1 pups, no statistically significant effects were observed on the total numbers of pups, number of live pups at birth, number of stillborn pups, or mean litter size at any time from birth to weaning. One F_1 control and one 1000-ppm group F_1 litter had one stillborn litter containing one pup each. One whole F_1 litter in the 1000-ppm group containing 14 pups died between day 0 and 4 of lactation, which resulted in a slightly lower pup viability index and litter survival index for this group. In addition, the lactation index was lower (92.4% vs 100% for control, N.S.) for the 1000-ppm F_1 group due to an increase in the number of pup deaths after day 4 of lactation; no F_1 pups in the control group died after day 4. The lactation index at 1000 ppm was outside the range of historical controls (see Table 7a). The sex ratio was not affected by treatment with the test material.

For F_2 pups, no statistically significant effects were observed on total numbers of pups, numbers of live pups at birth, numbers of stillborn pups, or mean litter size at any time from birth to weaning. The 100- and 300-ppm groups had one stillborn litter containing one pup each. Whole litter loss before day 4 occurred in the control (1 litter with 2 pups), 300-ppm group (2 litters with 1 and 14 pups), and 1000-ppm group (1 litter with 10 pups, 3 were still born). The pup viability index for 300-, and 1000-

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ppm groups was similar to that of the control group. Whole litter loss after day 4 occurred for one dam each in the 300- and 1000-ppm groups; each litter contained only eight pups because of culling on lactation day 4. As a result of the whole litter losses, the lactation index was decreased for the 300- and 1000-ppm groups compared with the control. The decreases were not statistically significant nor dose related; the lactation index at 300 ppm was notably lower than at 1000 ppm. The lactation index at 300 and 1000 ppm was also outside the range of historical controls. The litter survival index was also lower for 300 and 1000-ppm groups compared with the control group, but statistical significance was not achieved. The sex ratio of F_2 pups was not affected by treatment with the test material.

TABLE 7a. Mean litter size and viability during lactation of F_1 pups

Observation	Dietary concentration			
	Control	100	300	1000
F_1 Generation				
No. of litters with live pups	25 (24)	23 (23)	23 (23)	20 (19)
No. of pups live at birth	303	296	299	253
No. pups dead at birth	5	6	7	7
No. pups dead, day 0 - 21	8	21	13	35
Whole litter loss[a]	1	0	0	2
Mean litter size				
At birth	12.3±3.1[b]	13.1±2.5	13.3±2.5	13.0±3.7
Day 0[c]	12.1±3.2	12.9±2.6	13.0±2.4	12.6±3.8
Day 4 (precull)	12.1±2.2	12.1±2.6	12.6±2.5	12.6±3.0
Day 4 (postcull)	7.9±0.3	8.0±0.2	7.9±0.4	7.8±0.5
Day 7	7.9±0.3	7.9±0.5	7.9±0.5	7.8±0.5
Day 14	7.9±0.3	7.9±0.5	7.7±0.6	7.4±1.1
Day 21	7.9±0.3	7.9±0.5	7.7±0.6	7.2±1.4
Survival indices				
Pup viability (day 0-4)	96.4	94.9	96.6	89.9
Pup lactation[d] (day 4-21)	100.0	98.8	97.8	92.4
Historical Control (lactation)	F_1 pups: mean = 98.7%; range = 93.7-100.0% All pups: mean = 98.1%; range = 92.9-100.0%			
Litter survival index	100.0	100.0	100.0	94.7
Sex Ratio, Day 0 (M/F)	1.2	1.0	0.8	1.0

Data extracted from Tables 17 (pp. 163-166), 19 (p. 174), 20 (p. 176), and pages 264-271and 503, MRID 43178301.

[a]Includes still born litters.

[b]Mean±standard deviation

[c]Total number of pups born alive.

[d]Also called weaning index or day 4-21 pup survival index.

TABLE 7b. Mean litter size and viability during lactation of F_2 pups				
Observation	Dietary concentration			
	Control	100	300	1000
F_2 Generation				
No. of litters (with live pups)	25 (25)	24 (23)	23 (22)	22 (22)
No. of pups live at birth	307	317	263	271
No. pups dead at birth	5	12	7	8
No. pups dead, day 0 - 21	15	16	35	32
Whole litter loss[a]	1	1	4	2
Mean litter size				
At birth	12.5±3.2[b]	13.7±3.3	11.7±4.7	12.7±3.4
Day 0[c]	12.3±3.2	13.2±3.4	11.4±4.9	12.3±3.7
Day 4 (precull)	12.2±2.3	13.4±2.0	12.1±3.6	12.1±3.4
Day 4 (postcull)	8.0±0.0	8.0±0.0	7.6±1.4	7.7±0.9
Day 7	8.0±0.2	8.0±0.2	7.4±1.4	7.6±1.1
Day 14	7.8±0.8	7.8±0.7	6.9±1.7	7.4±1.1
Day 21	7.8±0.8	7.8±0.7	6.9±1.7	7.3±1.2
Survival indices				
Pup viability (day 0-4)	92.4	97.2	89.0	92.2
Pup lactation[d] (day 4-21)	97.4	97.8	88.1	91.3
Historical Control (lactation)	F_2 pups: mean = 97.8%; range = 92.9-100.0% All pups: mean = 98.1%; range = 92.9-100.0%			
Litter survival	96.0	100.0	86.4	90.9
Sex Ratio, Day 0 (M/F)	1.2	1.0	0.9	1.0

Data extracted from Table 17 (pp. 167-170), 19 (p. 175)Table 20 (p.176), and pages 272-279 and 503, MRID 43178301.
[a]Includes still born litters.
[b]Mean±standard deviation
[c]Total number of pups born alive.
[d]Also called weaning index or day 4-21 survival index.

2. Body weight

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Mean pup body weight data are presented in Table 8. No statistically significant differences were noted between mean pup weights of treated and control groups of either generation. High-dose group F_1 pups gained 7 or 8% less weight than controls after day 7 of lactation and for the entire lactation period; however, 1000-ppm group F_2 pups gained less weight (-10%) than controls only during the 14-21 day interval.

TABLE 8. Mean body weight and weight gain of F_1 and F_2 pups

Day of lactation	Dietary concentration (ppm)			
	Control	100	300	1000
F_1 Generation				
Pup weight (g)				
Day 0	6.3	6.3	6.4	6.2
Day 4[a]	9.6	9.9	9.6	9.3
Day 4[b]	9.6	10.0	9.6	9.3
Day 7	15.6	16.4	15.5	15.2
Day 14	31.3	33.1	31.2	29.7
Day 21	49.1	51.1	48.8	46.2
Pup weight gain (g)[c]				
Day 0-4	3.3	3.6	3.2	3.1
Day 4-7	6.0	6.4	5.9	5.9
Day 7-14	15.7	16.7	15.7	14.5
Day 14-21	17.8	18.0	17.6	16.5
Day 0-21	42.8	44.8	42.4	40.0
F_2 generation				
Pup weight (g)				
Day 0	6.1	5.9	5.9	6.3
Day 4[a]	9.1	8.9	8.8	9.4
Day 4[b]	9.2	8.9	8.7	9.5
Day 7	14.4	14.2	14.0	15.4
Day 14	29.6	30.1	30.0	31.2
Day 21	47.0	47.5	47.2	46.8
Pup weight gain (g)[c]				
Day 0-4	3.0	3.0	2.9	3.1
Day 4-7	5.2	5.3	5.3	5.9
Day 7-14	15.2	15.9	16.0	15.8
Day 14-21	17.4	17.4	17.2	15.6
Day 0-21	40.9	41.6	41.3	40.5

Data taken from Table 18 (pp. 171-172), MRID 43178301.
[a]Before standardization (culling)
[b]After standardization (culling)
[c]Calculated by the reviewer

3. Offspring postmortem results

a) Organ weights: Organs were not weighed.

b) Pathology

1) Macroscopic examination: No treatment-related findings were noted in weanlings of either generation receiving any dose of the test material. The kidney pelvis was moderately to extremely distended particularly in 100- and 300-ppm group F_2 weanlings.

2) Microscopic examination: Tissues from F_1 and F_2 pups were not examined microscopically.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that no adverse reproductive or systemic effects occurred at 100 ppm. At 300 ppm, a 9% reduction in weight gain for the entire treatment interval was observed in F_0 males. At 1000 ppm, mean weekly body weights were reduced in F_1 parental males over the entire treatment interval and weight gained over the entire treatment interval by F_0 and F_1 parental males was also reduced. The study authors concluded that F_0 and F_1 male fertility index and the pup survival index for lactation days 4-21 were reduced at 1000 ppm. According to the study author, the no-observed-adverse-effect level (NOAEL) was 100 ppm for parental toxicity and 300 ppm for reproductive toxicity.

B. REVIEWER'S DISCUSSION

Administration of MAA at doses of 100, 300, or 1000 caused no treatment-related effects on mortality or clinical signs in either F_0 or F_1 parental animals. Food consumption was in increased in F_0 and F_1 males of the 300 and 1000 ppm groups, F_0 females of the 1000 ppm group, and F_1 females of the 300 and 1000 ppm groups. Although increases in food consumption were increased, body weight and body weight gain were reduced by approximately 10% relative to control in males of the F_0 generation at 300 and 1000 ppm level and in males of the F_1 generation at 1000 ppm. These results of increased food consumption and decreased body weight gain are consistant with results from chronic feeding studies in mice (MRID no. 42173201) and rats (MRID no. 41669001) and are therefore considered treatment related. No effects on absolute body weights or body weight gain were observed in F_0 or F_1 females during the premating, gestation, or lactation periods. Food consumption was increased ($p > 0.05$) for female parental rats at 1000 ppm during the premating and gestation periods for both generations.

Postmortem examination of parental animals showed decreased absolute testes weights of 1000-ppm group of the F_0 generation and decreased prostate weight (absolute and relative) of 1000-ppm group F_1 males. No treatment-related gross or microscopic lesions were observed in F_0 or F_1 parental rats.

In conclusion, the parental LOAEL was 300 ppm (17.2 mg/kg/day) for male rats and 1000 ppm (77.6 mg/kg/day) for female rats based on increased food consumption with decreased body weight gain. The parental NOAEL is 100 ppm (5.6 mg/kg/day) for males and 300 ppm (22.5 mg/kg/day) for females.

The evaluation of reproductive performance showed no treatment related effects on sperm/spermatid count, morphology, or motility. The mating index was decreased in F_0 males of the 300 and 1000 ppm groups due to fewer animals who actually mated successfully (24 vs 28 in control). The mating index was actually higher in males of the F_1 generation. The fertility index of F_1 females of the 300 ppm group in addition to F_0 and F_1 males and females of the 1000 ppm group was reduced relative to control due to a reduced number of pregnant females. It is noteworthy that although the fertility indexes for the groups noted above are within the range of historical controls included with the study (76.2-100.0 for males; 71.4-100% for females), the actual values are on the low-end of this historical range (74.1-79.2%). These equivocal effects on reproductive performance are corroborated by Prukop and Savage (1986) who performed a one-generation reproduction study in mice gavaged with MSMA at 11.9 or 119 mg/kg every other day for 10 weeks. Decreased male fertility was observed at both doses compared to control. Only 50% of the females at 11.9 mg/kg/dose and none of the females at 119 mg/kg/dose became pregnant.

The reproductive LOAEL is 300 ppm (17.2 mg/kg/day) based on decreased fertility indexes in both sexes. The reproductive NOAEL is 100 (5.6 mg/kg/day).

Decreased lactation index compared to concurrent and historical controls was observed for 300 ppm F_2 pups and 1000-ppm group F_1 and F_2 pups. The decrease in the lactation index is due primarily to a whole litter loss at both dose levels. There were no treatment related effects on any other pup data. Body weights and body weight gain of F_1 and F_2 pups were comparable to control values throughout lactation. The number of pups that died between day 0-21 was increased in 300 ppm F_2 pups (35) and 1000-ppm group F_1 and F_2 pups (35 and 32, respectively) compared to control (8 and 15, respectively). Because of pup death, the litter survival index was reduced in these noted groups.

This equivocal data on pup death is corroborated by the Prukop and Savage (1986) study. In two of four females in the 11.9 mg/kg/dose group that produced litters, the mothers "did not build a nest, rarely huddled over the young, or retrieved them when they were separated from the young, all of which are maternal instincts." In these two litters, all of the young mice died with 2 to 3 days of birth. This postnatal litter loss parallels postnatal litter loss observed in rats of the guideline two-generation reproduction study.

Additional behavioral affects were observed in the Lopez and Judd (1979) where females treated with MSMA produced significantly smaller nests than did controls. Based on this literature evidence, it is plausible that the whole litter loss observed in the two-generation reproduction study is due to changes in the nurturing behavior of the mothers and not a direct toxic effect on the pups. Therefore, the increased pup death is not an indication of infant susceptibility but rather potential neurotoxicity in the females.

The offspring LOAEL is 300 ppm (17.2 mg/kg/day) based on increased pup death (day 0-21), reduced litter survival index, and decreased lactation index. The offspring NOAEL is 100 (5.6 mg/kg/day).

C. STUDY DEFICIENCIES

The analytical procedure used for measuring the concentration of MAA in the diet showed a lack of precision and the dietary concentrations were outside the range of acceptability for a large number of samples. Therefore, the weight-normalized-doses presented in this report may be quite different from the actual doses received by the animals.

D. REFERENCES

1. Lopez, JF and Judd, FW. 1979. Effect of sublethal dietary exposure of monosodium methanearsonic acid herbicide on the nest-building behavior of the white-footed mouse, Peromyscus leucopus. Bull. Environ. Contam. Toxicol. 23: 30-32.

2. Prukop, JA and Savage, NL. 1986. Some effects of multiple, sublethal doses of monosodium methanearsonic acid (MSMA) herbicide on hematology, growth, and reproduction of laboratory mice. Bull. Environ. Contam. Toxicol. 36: 337-341.