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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MAY 4 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 M. van Gemert 4/28/92
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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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.

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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 4/15/9
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.

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.

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 revieved 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).

Table 1

Stability of MAA 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 4[illegible], 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,

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.

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.

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	Group and Sex			
	Males*			
	1M	2M	3M	4M
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

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

	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
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 6

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

	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	137	140	155	102	104	108	115
%^^	--		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).

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).

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	4M	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]	319[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	181[c]	331[c]
102	140	158	185[a]	371[c]	145	149	163	276[c]
Mean[d]	137	141	176	341	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 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 each examination, the animals being selected prior to the commencement of treatment.

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 a (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 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).

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).

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	-	-	28	1.9	-	-	-
3F	72[a]	35[b]	-	-	30	1.8	-	-	-
4F	71[b]	35[b]	-	-	24[b]	1.6[b]	-	-	-
6 Months									
1M	64	--	2.5	-	-	-	-	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	-	2.3	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, $p < 0.001$

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

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).

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Table 13

Mean Urinalysis Parameters[1]

Group	1M	2M	3M	4M	1F	2F	3F	4F
				Group and Sex				
				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	7.9	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[c]	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

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* cervical/ mesenteric	X sciatic nerve
X jejunum*		X pituitary*
X ileum*	X spleen*	X eyes* & optic nerve*
X cecum*^^	X thymus*	Glandular
X colon*^^	Uxogenital	XX adrenals*
X rectum*^^	XX kidney*^^	X parathyroids*^^
XX liver*	X urinary bladder	XX thyroids*^^
X pancreas*	XX testes(b)*	Other
Respiratory	X prostate*	X bone*
X trachea*	X seminal ves.	X skeletal muscle*
X lung*^^	X ovaries(a)*	X skin*
	X uterus*	X unusual lesions*
		- 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).

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[b]	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[b]	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, $p < 0.001$

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.

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.

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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.

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.

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	0/5[illegible]	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.

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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 dehydrated 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).

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).

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.

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).