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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

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Tox Branch II; HED (7509C) Marcia van Gemert 7/25/94

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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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.

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.

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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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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

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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.

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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®.

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.

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(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	Cholesterol* [Chol]
Aminotransferase* [ALT]	Triglycerides
Serum Aspartate	
Aminotransferase*	
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.

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.

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Organ Weights

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

*Required by Guidelines

(3) Histopathology

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

Histopathology

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

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

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 Conditions[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	2	0	3	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	0	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 anoretic during this approximate time period.

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).

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].

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 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.

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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).

Bilirubin in the male was marginally decreased at all dose levels on week 12, at 35 mg/kg/day on week 26 and at all dose levels on week 51 where at 2 mg/kg/day statistical significance ($p<0.05$) was seen. In females, decreases were seen at all dose levels at Weeks 26 and 51 with statistical significance ($p<0.05$) at 2 and 35 mg/kg/day vs. the respective concurrent controls at week 51. No dose-relation-ship was seen at any time period during the study (Table 7, 8).

Calcium levels in the female were reduced at 8 mg/kg/day after 12 weeks [$p<0.001$] of treatment, at 8 and 35 mg/kg/day at 26 weeks [$p<0.05$ and $p<0.01$, respectively] and at 35 mg/kg/day prior to termination [$p<0.001$] compared to the concurrent controls. In the male at the 35 mg/kg/day, decreases occurred at week 51 [$p<0.01$] compared to the concurrent controls (Table 7, 8).

Total protein was decreased in the female at all dose levels and time periods [except Weeks 12 and 26 at 2 mg/kg/day] and showed statistical significance at 2 mg/kg/day [$p<0.05$] at week 51 and at 35 mg/kg/day [$p<0.05$ to 0.001] at all time periods vs. the concurrent controls. Decreases in albumin occurred at 35 mg/kg/day at all time periods and were statistically significant at 12 ($p<0.05$) and 51 ($p<0.01$) weeks vs. the concurrent controls (Table 9).

Gamma-globulin in the male showed sporadic increases at 2 [$p<0.05$] mg/kg/day at 12 weeks and 26 weeks and at 35 mg/kg/day at 12 [$p<0.01$] weeks. Changes in the other globulin fractions were similar to the concurrent controls at all time periods (Table 10).

The various globulin fractions in the female were all decreased at 35 mg/kg/day at all time periods vs. the concurrent controls. The $\alpha 2$ and gamma-globulin fractions showed dose related decreases at weeks 26 with the $\alpha 1$ and gamma-globulin fractions showing statistically significant decreases ($p<0.01$, $p<0.05$, respectively) vs. the concurrent controls. At week 51 the β-globulin fraction showed a dose related decrease (Table 10).

Other changes observed in the clinical chemistry parameters were considered either sporadic in nature or not related to the administration of the test compound.

(3) Urinalysis

After 12 weeks of treatment, treatment related decreases in urine volume occurred in male dogs at all dose levels vs. the concurrent control; these values coincided with a dose related increase in the specific gravity at 2, 8, and 35 mg/kg/day ($p<0.05$) vs. the concurrent control (Table 11).

At week 25 urine output was decreased in males at 8 and 35 mg/kg/day vs. the concurrent control but specific gravity was not

affected. At week 52, a marginal decrease in the urinary volume output vs. the control males was seen at 35 mg/kg/day with a statitically significant ($p<0.01$) increase in the specific gravity vs. the concurrent control (Table 11).

In the females at 35 mg/kg/day, at all time periods, urinary volume was marginally decreased, and specific gravity was marginally increased vs. the concurrent controls [except for the specific gravity at week 51]. No statistical significance was seen (Table 11).

J. Pathology

(1) Organ Weight

No statistically significant differences occurred in the absolute organ weight values of treated groups as compared to the respective Controls (Table 12).

In the male, the mean adrenal/body weight ratio was increased at 35 mg/kg/day [1.094; $p<0.05$ vs. 0.847 in the Control] (Table 12).

The mean heart/body weight ratio at 35 mg/kg/day showed statistically significant [$p<0.05$] increases in the male [0.869 vs. 0.739 in the Control]. In the female, a dose related increase occurred showing statistical significance [$p<0.05$] at 35 mg/kg/day [0.828 vs. 0.650 in the Control] (Table 12).

In the male, the mean liver/body weight ratio was increased at 35 mg/kg/day [2.839 vs. 2.598 in the Control; $p<0.05$]. No changes were seen in the liver to brain weight ratio or in the liver weight following analysis of covariance corrected for body weight (Table 12).

No changes were seen in the female liver/body weight ratio except at 2 mg/kg/day where a statistically significant [$p<0.05$] decrease occurred. Decreases [not statistically significant] were seen in the liver weight/brain weight ratio at all dose levels. However, using the analysis of covariance of liver weights corrected for body weight showed a decrease [not statistically significant] only at 2 mg/kg/day (Table 12).

A dose related increase occurred in the mean kidney weight/body weight ratio for both sexes culminating in statistical significance [$p<0.05$] in the female at 8 [0.460] and 35 [0.462] mg/kg/day vs. the Control [0.392] (Table 12).

The mean brain weight/body weight ratio in the females showed a dose-related increase vs. the controls but statistical significance was not seen at any dose level. The analysis of covariance of brain weight was not remarkable for either sex or dose level (Table 12).

Table 6. Hematology Parameters*

Dose [mg/kg/day]	Hematology Parameters							
	Males				Females			
	PCV [%]	Hb [gm%]	RBC $10^6/\mu l$	ESR [mm/hr]	PCV [%]	Hb [gm%]	RBC $[10^6/\mu l]$	ESR [mm/hr]
	Week 0							
0	42.2	14.4	6.00	0.0	43.8	15.1	6.16	0.6
2	40.2	14.0	5.79	0.0	46.0	15.7	6.22	0.0
8	41.0	14.1	5.77	0.0	45.2	15.3	6.28	0.0
35	41.8	14.3	5.79	0.8	43.8	15.0	6.29	0.0
	Week 12							
0	46.6	15.8	6.44	1.0	48.4	16.7	6.92	0.4
2	46.4	15.8	6.41	1.2	46.8	16.2	6.54	0.2
8	46.6	16.0	6.37	0.6	46.8	16.3	6.60	0.6
35	44.2	15.1	6.17	5.2	47.6	16.4	6.65	0.2
	26 weeks							
0	45.4	15.7	6.49	0.4	48.0	17.5	7.55	1.0
2	46.6	16.2	6.68	0.8	48.2	17.6	7.43	4.2
8	46.6	16.4	6.60	0.8	47.4	17.4	7.41	3.8
35	43.8	15.4	6.23	1.8	43.8	16.1	6.97	0.4
	51 Weeks							
0	50.2	17.5	7.17	0.4	50.6	17.8	7.33	1.0
2	51.2	17.9	7.38	0.4	53.4	18.6	7.47	0.2
8	50.8	18.0	7.10	0.2	49.2	17.3	7.04	0.8
35	47.6	16.8	6.82	1.6	49.4	17.3	6.92	0.0

*Adapted from original report, p. 118 to 125.

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Table 7. Clinical Chemistry Values in Males[a]

Dose [mg/kg/day]	Clinical Chemistry Values in the Male						
	ALP IU/l	LDH IU/l	ALT IU/l	CPK IU/l	Chol mM/l	Ca mM/l	Bili µM/l
	Week 0						
0	80	45	33	156	3.26	2.31	5.40
2	90	109	38	186	3.24	1.21	4.80
8	81	68	27	154	3.90	1.95	5.00
35	79	91	34	148	3.32	2.55	3.00
	Week 12						
0	36	158	24	183	4.02	2.66	2.40
2	49	171	28	196	3.77	2.58	1.40
8	40	159	24	170	4.36	2.62	1.60
35	43	223	31	249	3.83	2.66	2.00
	Week 26						
0	84	306	26	153	3.12	2.75	2.60
2	134*	495	26	158	2.92	2.78	2.80
8	98	402	24	157	3.53	2.70	2.60
35	105	591	30	231*	3.09	2.77	2.40
	Week 51						
0	77	449	30	76	3.35	2.68	2.20
2	126**	507	32	63	2.90	2.59	1.60*
8	88	292	28	74	3.71	2.68	2.00
35	104	490	22*	100	2.84	2.52**	1.80

[a]Adapted from original report, p. 126 to 137.
*p<0.05; **p<0.01

Table 8. Clinical Chemistry Values in Females*

Dose [mg/kg/day]	Clinical Chemistry Values in Females						
	ALP IU/l	LDH IU/l	ALT IU/l	CPK IU/l	Chol mM/l	Ca mM/l	Bili µM/l
	Week 0						
0	69	69	30	144	3.24	2.56	3.80
2	71	74	30	154	3.43	1.18	4.00
8	63	62	30	124	3.20	2.48	4.00
35	[illegible]	53	31	156	3.26	2.52	4.60
	Week 12						
0	35	112	24	173	4.57	2.75	1.00
2	40	94	25	175	5.00	2.64	1.20
8	32	98	22	153	4.12	2.42**	1.20
35	36	161	21	196	3.71	2.64	1.20
	Week 26						
0	83	468	22	177	4.38	2.83	2.60
2	91	345	25	161	4.19	2.78	2.20
8	83	284	22	123	3.54	2.66*	2.20
35	81	262	18	178	3.30	2.63**	2.20
	Week 51						
0	69	381	23	67	4.65	2.73	3.40
2	73	591	25	73	3.88	2.66	2.40*
8	81	567	22	83	4.19	2.63	2.60
35	89	403	15*	85	3.66*	2.49**	2.20*

*Adapted from the original report, p. 126 to 137.
*p<0.05; **p<0.05; ***p<0.001

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Table 9. Total Protein and Albumin in Females*

Weeks on Test	Total Protein [gm/l]				Albumin [gm/l]			
	Dose [mg/kg/day]							
	0	2	8	35	0	2	8	35
0	59.0	55.2	58.4	53.8	32.7	31.1	32.1	29.4
12	69.6	69.6	67.6	65.2*	36.4	34.7	37.2	33.8*
26	74.2	74.4	70.6	63.6**	37.0	38.8	40.1	36.8
51	78.0	71.6*	75.6	65.8***	41.4	39.4	39.9	33.9**

*Adapted from the original report, p. 127 to 136.
*p<0.05; **p<0.01; ***p<0.001

Table 10. Globulins Fractions Values*

Dose [mg/kg/day]	Globulin Fractions [gm/l]							
	Males				Females			
	Week 0							
	α1	α2	β	gamma	α1	α2	β	gamma
0	3.45	7.80	11.82	2.68	3.45	7.42	12.38	3.01
2	2.79	8.66	11.51	3.10	2.71	7.71	10.92	2.78
8	2.90	8.34	10.63	2.86	3.16	8.49	11.59	3.08
35	3.02	9.96	13.27	2.72	3.00	8.31	9.98	3.09
	Week 12							
0	3.67	2.40	17.65	2.84	3.77	11.47	13.42	4.59
2	3.08	12.57	19.00	4.70*	3.77	12.72	14.89	3.56
8	3.17	13.38	18.59	3.86	3.07*	11.00	12.28	4.08
35	3.84	11.79	17.31	5.30**	3.51	10.32	13.87	3.71
	26 weeks							
0	2.36	11.00	16.63	3.66	3.27	13.01	16.35	4.55
2	2.44	10.60	17.95	6.15*	3.46	11.89	16.71	3.53
8	2.06	11.26	16.58	3.98	2.71	10.78	13.64	3.43
35	2.68	10.97	16.13	4.95	1.99**	10.22	12.65	1.92*
	51 Weeks							
0	5.04	9.15	17.89	5.20	3.81	10.51	17.59	4.71
2	5.59	9.72	18.08	5.31	3.74	8.24	15.16	5.83
8	6.18	9.30	18.32	7.33	5.22*	9.69	14.57	6.28
35	5.53	9.90	15.96	5.86	4.86	9.12	13.80	4.11

*Adapted from original report, p. 127 to 136.; *p<0.05; **p<0.01

Table 11. Urinalysis[a]

Dose [mg/kg/day]	Males		Females	
	Volume [ml]	Specific Gravity	Volume [ml]	Specific Gravity
	0 Weeks			
0	364	1.026	244	1.028
2	268	1.036	168	1.036
8	254	1.037	248	1.033
35	384	1.025	382	1 024
	12 Weeks			
0	230	1.041	170	1.051
2	180	1.046	170	1.052
8	165	1.055*	185	1.050
35	170	1.058*	150	1.059
	25 Weeks			
0	242	1.044	188	1.046
2	296	1.041	170	1.049
8	222	1.043	180	1.039
35	200	1.049	120	1.057
	52 Weeks			
0	174	1.050	150	1.046
2	180	1.055	200	1.050
8	180	1.052	160	1.044
35	156	1.064**	140	1.046

[a]Adapted from original report, p. 138 to 145.
*p<0.05; **p<0.01

Table 12. Organ Weight Parameters[a]

Dose [mg/kg/day]	Brain	Heart	Liver	Kidney	Adrenals
	Males				
0	78.42 [0.569]	101.48[b] [0.739][c] 1.307[d]	357.0 [2.598] 4.602	60.60 [0.441] 0.779	1.166 [0.8[illegible]] 0.01[illegible]
2	80.84 [0.621]	109.00 [0.839] 1.358	349.4 [2.692] 4.356	61.64 [0.477] 0.777	1.182 [0.912] 0.015
8	75.60 [0.564]	106.66 [0.799] 1.413	350.6 [2.617] 4.638	60.94 [0.457] 0.806	1.070 [0.799] 0.014
35	73.76 [0.618]	103.78 [0.869*] 1.415	338.6* [2.839*] 4.608	60.64 [0.506] 0.824	1.302 [1.094*] 0.018
	Females				
0	77.36 [0.550]	93.52 [0.650] 1.235	395.8 [2.741] 5.232	56.04 [0.392] 0.735	1.542 [1.065] 0.020
2	76.74 [0.584]	93.92 [0.697] 1.229	341.2* [2.476*] 4.501	60.54 [0.442] 0.794	1.538 [1.101] 0.020
8	71.76 [0.609]	86.56 [0.716] [1.207]	345.4 [2.879] 4.844	55.18* [0.460*] 0.772	1.522 [1.241] 0.021
35	81.20 [0.665]	102.78 [0.828*] 1.262	346.2 [2.749] 4.256	57.24* [0.462*] 0.704	1.706 [1.396] 0.021

[a]Adapted from the original report, p. 146, 147 and Amendment p. 15, 16.
[b]Organ Weight (gm)
[c]Organ to body weight ratio (%); Adrenals [% x 100]
[d]Organ to brain weight ratio (%)
*p<0.05

(2) Gross Pathology

A single male animal showed reduction in the abdominal fat pads at 35 mg/kg/day. There were no other differences in gross pathology suggestive of toxicity.

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(3) Histopathology

All control and treated groups in both sexes showd variable hepatocytic glycogen contents depletion which was represented by perinuclear hepatocyte pallor [empty spaces]. No dose relationship or trend existed compared to the controls. In a single female dog at 35 mg/kg/day, the glycogen had been completely depleted.

The incidence of female animals showing no corpora lutea were increased [3/5] at 35 mg/kg/day vs. the Control [0/5].

In the female, the incidence of a moderate tubular nephrosis [characterized by small vacuolation in the epithelial cells] were increased at 8 [1/5] and 35 mg/kg/day [2/5] vs. the Control [0/5]. Deviation: A signed and data histopathology report was not included in this report.

V. DISCUSSION:

During the physical examination, animals with dull/lusterless coats were observed more frequently in treated vs. the control animals. The number of thin male animals appeared to correlate at 35 mg/kg/day [but not at 2 mg/kg/day] with the decrease in the weight parameters. Correlation was not seen in the female [except for the single cachectic female at 35 mg/kg/day].

Clinical signs associated with the administration of the test compound were vomiting, diarrhea, excessive salivation, a decreased incidence of estrus and sporadic anorexia at the high dose vs. the control. A greater incidence of diarrhea and excessive salivation occurred in the female compared to the male while a greater incidence of vomiting occurred in the male compared to the female. The incidence of excess salivation was not always associated with the administration of the test compound.

Marked and statistically significant decreases in the mean body weight gain seen at 35 mg/kg/day in both sexes and at 8 mg/kg/day in the females, at all time periods, were considered to be related to the administration of the test compound. The minimal weight decreases in the male at 2 and 8 mg/kg/day were considered to be of little toxicological significance.

The weight losses seen in both sexes was also correlated with the vomiting, diarrhea and the anorexia. One female at the high dose level that presented anorexia was also cachectic. It is unknown from the presented data whether the weight changes were due to the vomiting and/or diarrhea or through some other mechanism.

A slight (<5%) decrease in food consumption was seen in the females at 8 and 35 mg/kg/day, which was related to the weight loss seen at these dose levels.

The hematology and clinical chemistry alterations were considered to be minimal. Lowered cholesterol at 35 mg/kg/day in the females appeared related to the reduced calcium and protein levels.

Increased urine specific gravity correlated with a decreased urine output in the male. Females showed a decreased volume of urine at 35 mg/kg/day. These changes were considered to be treatment related.

The increases in the organ to body weight ratio in the male [adrenals, heart, liver, kidney] and female [heart, kidney, brain] appeared to be caused by the loss of body weight as evidenced by the analysis of covariance corrected for body weight supported by no differences in the organ weight to brain weight ratio. Sporadic decreases in the relative liver weight parameters of the females at 2 mg/kg/day were not related to treatment.

Gross pathology in the single high-dose animal male animal showing a reduction in the abdominal fat pads could be correlated with the loss of body weight and/or debility. Likewise, histopathology seen in the liver, kidney and ovary was also caused by a loss of body weight and/or debility as noted below.

A single female at 35 mg/kg/day showed depletion of glycogen stores [as evidenced by perinuclear pallor]. Glycogen is normally found in the cytoplasm of the hepatocyte in well nourished individuals. This particular animal presented weight loss and vomiting possibly explaining the reduction in the hepatic glycogen reserves.

The decrease in the estrus incidence at the 35 mg/kg/day (2/5), appeared to correlate with the absence of corpora lutea seen in the histopathology examination. Anestrus will develop secondary to debility or marked loss of weight.

Female dogs at 35 mg/kg/day (2/5) and 8 mg/kg/day (1/6) evidenced fatty vacuolation of the kidney confined to the cortico-medullary junction. The authors note that starved animals frequently show this lesion.

Based on the above results, the LOEL was 8 mg/kg/day and the NOEL was 2 mg/kg/day.

Adequacy of the Dose Levels to Assess Chronic Toxicity

In this chronic dog study, the dose levels used were judged sufficient to access the chronic toxicity of the test compound as evidenced by the clinical signs and weight changes at 8 and 35 mg/kg/day.

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

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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/006/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.

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

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

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

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.

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 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).

Table 3. Food Consumption Dr q 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 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 [%] # Aborted [%] # Non pregnant [%]	 0 0 2	 2 0 0	 0 0 0	 0 0 0	 1 0 0	 0 2 [15.4] 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 (#) Live Fetuses (Mean±SD)	97 8.1±2.9	98 8.2±2.4	124 8.9±2.6	95 7.3±1.4	112 8.6±1.9	80 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 [♂/♀] (% Males)	49/48 50.5	56/42 57.1	55/69 44.4	53/42 55.8	66/46 58.9	41/39 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).

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).

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,c]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 3.3% 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).

Table 6. Summary of Selected Fetal Variations[a]

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

[a]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].

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 1[illegible] 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].

(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.