10-3-92
SECTION HEAD

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

009775

OCT 0 3 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

Subject: Review of Toxicology Study with Cacodylic Acid to support registration of test substance. (Toxchem Number 133, DP Barcode number: D180029)

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 9/29/92
Tox. Branch II, Review Section IV
HED (H7509C)

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

THRU: Elizabeth Doyle, Ph.D., Section Head E.A. Doyle 9/29/92
Tox. Section II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief M van Gemert 9/29/92
Tox. Branch II, HED (H7509C)

ACTION REQUESTED: Review of supplementary data (MRID 423625-01, Cacodylic Acid; Toxicity in Dietary Administration to Mice for 13 Weeks: A Preliminary Study) to upgrade mouse oncogenicity study (MRID 419146-01) for reregistration requirements.

REVIEW

The Agency has reviewed the original oncogenicity report (MRID 419146-01), the associated Data Evaluation Report and the reregistration data submitted under MRID 423625-01 (82-1 Subacute Toxicity - Mouse).

The Agency has established that in the mouse oncogenicity study (MRID 419146-01) there was, indeed, a significant decrease in body weight gain in male (≥10%) but not female animals throughout the study; this fact was noted by the registrant.

It is the Agency's position that a dose be used in an oncogenicity study that decreases the rate of weight gain by ≥10%, the so called Maximum Tolerated Dose (MTD), unless there is justification that this dose cannot be used because of the potential for the effect or lesion to decrease survival on prolonged exposure.

If animals are not dosed at the MTD, the study is considered supplementary. In the mouse oncogenicity study, the MTD was reached in the males but not in the females.

A 13 week study usually defines the toxicity profile (with the exception of carcinogenicity and other specialized toxicities) and can be used to estimate a working MTD for use in a oncogenicity study. In the 13 week study (MRID 423625-01), at the 500 ppm dose level, a decrease in the rate of body weight gain of about 9% occurred in males versus the control. No decrease in the rate of body weight gain was noted on the females. A decrease of less then 10%, however, is deemed by the Agency to be insufficient to define a MTD.

At the 2000 ppm dose level, however, a clear decrease in the rate of body weight gain was noted, i.e., 30% in the males and 15% in the females versus the controls.

The conclusion is, therefore, that a dose level lower than 2000 ppm, but higher than 500 ppm, i.e., 1000 ppm should have been chosen for the high dose level in the mouse oncogenicity study to give some insurance that the dose would have met or exceeded the MTD.

The Agency is also of the opinion that the pathology noted namely, vacuolar degeneration of the superficial transitional epithelium in the urinary bladder at 500 and 2000 ppm would not result in decreased survival on prolonged exposures and should have been of secondary importance for MTD dose selection.

The same can be said of the other parameters cited, such as increased water consumption and relative organ weight change at the 500 ppm dose level.

The Agency is also of the opinion that while certain classes of compounds toxicologically tend to have similar actions, the toxicity of the arsenic compounds is so unique that small molecular changes can profoundly affect the toxicity of the test compound; cacodylic acid, therefore, cannot be compared to other arsenic compounds.

The Agency is in agreement and accepts the rebuttal concerning the exclusion of certain individual organ weights from the means and the explanation of why some clinical pathology were not reported in the oncogenicity study.

It is suggested that another study with a similar protocol be run using a control and high dose level of approximately 1000 ppm; in

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Reviewed by Steven L. Malish, Ph.D. S.L. Malish 9/29/92
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 9/29/92
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE:	82-1 Subchronic Toxicity - Mouse
MRID NO:	423625-01
TEST MATERIAL:	Cacodylic Acid
SYNONYMS:	dimethylarsinic acid, cacodylic acid
SPONSOR:	Luxembourg Industries (Pamol) Ltd. PO Box 13 Tel-Aviv, Israel
TESTING FACILITY:	Life Science Research Israel, Ltd. PO Box 139, Ness Ziona, 70 451, Israel
LAB STUDY NO.:	PAL/013/CAC
TITLE OF REPORT:	Cacodylic Acid Toxicity in Dietary Administration to Mice for 13 Weeks: A Preliminary Study
AUTHORS:	S. Crown, A. Nyska
REPORT ISSUED:	April, 1992

CONCLUSIONS:

Six groups of 12 mice/sex of the B6C3F1 strain were administered cacodylic acid at concentrations admixed in the feed at 0 (Control), 5, 50, 500, 2000 and 5000 ppm for 13 weeks.

Animals at 5000 ppm died or were sacrificed in extremis within 8 weeks of the start of the study. No mortality was noted at the lower dose levels.

At 2000 ppm dose level a decrease in the rate of body weight gain of approximately 30% in males and 15% in females occurred together with a 17% increase in food consumption in the females versus the controls. A decrease in the efficiency of food conversion was seen in the males at the 500 and 2000 ppm dose levels. In females, a 17% and 28% increase in water consumption was seen, respectively, at the 500 and 2000 ppm dose levels throughout the study.

In males at 2000 ppm, the brain, liver, kidneys, testes and spleen showed a decrease in organ weights compared to the controls. Females showed an absolute brain weight decrease and a relative brain weight increase at the 500 and 2000 ppm dose level.

Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in both the 500 and 2000 ppm dose levels in both sexes.

MTD: males - <433 mg/kg/day but >102 mg/kg/day (<2000 but >500 ppm)
females - <604 mg/kg/day but >128 mg/kg/day (<2000 but >500 ppm).

NOEL: males - 10 mg/kg/day (50 ppm)
females - 13 mg/kg/day (50 ppm)

LOEL: males (pathology) - 102 mg/kg/day (500 ppm)
females (pathology) - 128 mg/kg/day (500 ppm)

CLASSIFICATION

Core: Supplementary - not upgradeable

The study does not satisfy the guideline requirement (82-1) for a subchronic toxicity study.

The study is deficient with regard to the required hematology and clinical chemistry parameters.

QUALITY ASSURANCE

A quality assurance statement was included.

FLAGGING CRITERIA

The following statement was included on p. 5A of the original report and was signed and dated by an authorized representative of the Company.

"I have applied for criteria of 40CFR 158.34 for flagging studies for potential adverse effects to the results of the attached studies. This study neither meets nor exceeds any of the applicable criteria." [The reviewer agrees with this statement.]

MATERIALS:

Test Compound

Common name:	cacodylic acid
Label:	Cacodylic acid, 500 gr. Pamol Ltd. Arad.
Batch No:	1007
Trade Name:	dimethylarsenic acid, cacodylic acid
Chemical name:	dimethyl hydroxyarsine oxide
Purity:	99.5%[1]
Description:	white crystalline solid
Storage:	room temperature

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[1]Adapted from p. 59 of the original report. Analysis performed by sponsor.

Analysis of Formulated Diets

Stability

Stability was determined from the trial mix prepared prior to commencement of the study. Samples (5, 500, or 5000 ppm) were analyzed 7, 14 and 21 days after mixing diet.

No degradation was noted at any of the time periods when compared to the required values.

Homogeneity

Homogeneity of cacodylic acid 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 in all groups and analyzed for cacodylic acid.

The mean of all groups was within 10% of the required value.

Achieved Concentration

Checks were made to verify the test material content on Weeks 5, 9 and 13 of the study. Concentrations on week 5 were within -9% of the nominal value; on week 9 within -14% of the nominal value and on week 13 within -0.4% and 22% (50 ppm) of the nominal value. The reason for the large variation at 50 ppm is not known at the present time. [The reviewer does not believe that a variation at the low-intermediate dose would infer with the interpretation of the study].

Test Animals

Species: mice (pathogen free)
Strain: Charles River B6C3F1
Age: 4 weeks of age at initiation of the study
Weight: males - 16.3-21.8 gm; females - 12.7-17.8 gm at study initiation
Source: Charles River Breeding Laboratories, UK

STUDY DESIGN:

Animal Assignments

Animals were acclimatized for 12 days and housed 4 per sex in each cage.

Seventy two (72) animals per sex were randomly assigned to five (6) test groups and administered 0 ppm, the control (Group 1), 5 (Group 2), 50 (Group 3), 500 (Group 4), 2000 (Group 5) and 5000 ppm (Group 6) ad-mixed in the feed for 13 weeks (Table 1).

Table 1

Animal Test Group Assignments[1]

Group	Treatment	Dietary Conc. (ppm)	Animals on Test (M/F)
1	Control	0	12/12
2	cacodylic acid	5	"
3	cacodylic acid	50	"
4	cacodylic acid	500	"
5[2]	cacodylic acid	2000	"
6[2]	cacodylic acid	5000	"

[1]Adapted from original report p. 12.
[2]Deleted from the subsequent tables in the report because of the death of all animals.

Diet

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

Diet preparation

An initial premix was followed by serial dilutions of the basal diet. All test diets were prepared biweekly.

Water Supply

Drinking water obtained from the public water supply was sterilized and then supplied to the cages via polyethylene bottles and stainless steel sipper-tubes.

Statistics

Body weight, food consumption, water consumption, organ weight and ratio differences were compared to the controls by the Students' t-test using pooled intragroup error variance.

METHODS AND RESULTS:

Observations

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

The 5000 ppm dosed animals showed "diarrhea, emaciation, tremors, hunching, hyperthermia and generalized debility." No signs due to treatment were noted in the lower dose animals throughout the study.

Mortality

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.

Animals treated with the 5000 ppm dose level of cacodylic acid died or were sacrificed in extremis within 8 weeks of commencement. Females appeared to succumb more quickly to the effects of test compound versus the males - Week 2 (died 1 M, 4F), week 3 (1 M, 6 F), week 4 (5 M, 1F), week 5 (1F), week 6 (2 M), week 7 (3 M).

Body Weight

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

At the 2000 ppm dose level in the males, an absolute weight decrease of 15.1% was noted on week 13 when compared to the control. The body weight gain decreased 30.3% when compared to the control (Table 2).

Male animals at the 500 ppm dose level showed statistically significant but negligible decreases in the absolute weight (<8%) and rate of weight gain in male animals from weeks 4 through 10 versus the control. At week 13, the decrease in absolute weight

compared to the control was 2.8% while the change in body weight decrease versus the control was 9.1%. These weight changes were considered to be toxicologically unremarkable (Table 2).

Female animals at the 2000 ppm dose level showed a statistically significant decrease in body weight starting on week 2 and continu -ing to the end of the study. At week 13, the change in the body weight gain versus the control was a -14.8%. No toxicologically significant absolute weight gain ($p<0.05$) was seen (Table 3).

Table 2

Mean Body Weights (gm) of Male Animals at Selected Time Intervals Throughout the Study[1]

	Dose(ppm)				
Weeks	0	5	50	500	2000
0	19.2	17.8[a]	18.7	19.5	18.3
1	21.6	20.6	21.5	20.6	19.0[c]
2	23.0	22.7	23.5	22.7	20.0[c]
4	26.7	25.7	26.0	24.6[b]	21.1[c]
6	28.4	28.3	28.7	26.7[a]	23.3[c]
8	30.0	29.5	30.2	28.2[a]	24.3[c]
10	31.5	31.4	31.9	29.9[a]	26.4[c]
13	32.4	32.6	32.9	31.5	27.5[c]
Abs. Wgt.[2]					
%[3]	--	0.6	1.5	-2.8	-15.1
Change[4]	13.2	14.8	14.2	12.0	9.2
%[3]	--	12.1	7.6	-9.1	-30.3

[1]Adapted from original report, p. 32. Twelve (12) animals at each point
[2]Absolute weight (gm) compared to the control at 13 weeks.
[3]Percentage difference
[4]Change in weight gain (gm) compared to the control from 0 thru 13 weeks
[a]Significantly different from controls, $p<0.05$.
[b]Significantly different from controls, $p<0.01$.
[c]Significantly different from controls, $p<0.001$.

Table 3

Mean Body Weights (gm) of Female Animals at Selected Time Intervals Throughout the Study[1]

	Dose(ppm)				
Weeks	0	5	50	500	2000
0	15.1	14.9	15.5	15.9	15.2
1	16.6	16.6	16.6	16.4	15.6[a]
2	17.6	17.5	17.7	17.1	16.5[a]
4	19.0	19.2	19.5	19.2	17.2[c]
6	20.9	21.0	21.2	20.6	18.9[c]
8	21.5	21.3	21.8	21.1	19.1[c]
10	23.1	22.4	22.8	22.3	21.0[c]
12	23.3	23.5	23.5	22.6	22.0[b]
13	23.9	23.8	24.5	23.5	22.7[b]
Abs. Wgt.[2]					
%[4]	--	0.4	2.5	2.5	5.0
Change[3]	8.8	8.9	9.0	8.6	7.5
%[4]	--	1.1	2.2	-2.2	-14.8

[1]Adapted from original report, p. 32. Twelve (12) animals at each point.
[2]Absolute weight (gm) compared to the control at 13 weeks.
[3]Change in weight gain (gm) compared to the control at 13 weeks.
[4]Percentage difference
[a]Significantly different from controls, $p<0.05$.
[b]Significantly different from controls, $p<0.01$.
[c]Significantly different from controls, $p<0.001$.

Food Consumption

Food consumption in the female animals showed an increase of approximately 17% throughout the study at the 2000 ppm dose level when compared to the control group at week 13. No effects were seen at the lower dose levels or in the male animals.

Efficiency of Food Conversion

Efficiency of food conversion (ratio between body weight change to the weight of food consumed) was calculated each week of the 13 week study.

In males, the efficiency of food conversion was decreased at 500 and 2000 ppm by 33% compared to the control. No effects were noted at the lower dose levels in the males or in the females at any dose level.

Compound Consumption

Compound consumption expressed as mg/kg/day was calculated for each group/sex (Table 4).

Table 4

Mean Compound Consumption for Weeks 1 thru 13[a,b,c]

Gr.	Dose[d] (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
1	0	0.0	0.0
2	5	1.0	1.3
3	50	9.7	12.5
4	500	101.9	127.5
5	2000	433.0	604.3

[a]Adapted from original report, p. 37.
[b]Calculated from the average food consumption and body weight at all time intervals during the study.
[c]Mean compound consumption calculated by reviewer.
[d]Nominal dose in each group
Gr. denotes group

Water Consumption

The amount of water imbibed by each cage of rats was recorded weekly for 13 weeks. Group means were calculated at each time period.

Female animals showed a dose related increase in water consumption which ranged from 11% at the 5 and 50 ppm dose level to 17 and 28% at the 500 and 2000 ppm dose level, respectively. Weekly mean values showed statistical significance starting at week 1 at 2000 ppm while at 500 ppm statistical significance was seen by week 6 (Table 5).

No change in water consumption was seen in the males when compared to the control (Table 5).

Table 5

Mean Water Consumption (ml/animal/week) at Selected Intervals Throughout the 13 Week Study[1,2]

Dose (ppm)

	Males					Females				
Week	0	5	50	500	2000	0	5	50	500	2000
2	40	42	40	42	40	33	37	36	37	39[a]
4	51	49	47	46	43	36	39	38	42	50[a]
6	49	55	54	49	45	37	43[a]	40	41[a]	46[c]
8	46	53	51	43	45	38	43[b]	41	42[a]	46[c]
10	46	55	50	46	47	39	41	41	50[a]	50[a]
12	47	59	54	43	45	39	42	41	43	50[b]
13	47	57	52	42	43	39	41	41	46[b]	50[c]
Mean[d]	47	52	49	45	44	36	40	40	42	46
%[e]	--	11	-4	4	-6.4	--	11	11	17	28

[1]Adapted from original report, p. 38 and 39.
[2]Mean of 3 animals at each evaluation time.
[a]Significantly different from controls, $p<0.05$.
[b]Significantly different from controls, $p<0.01$.
[c]Significantly different from controls, $p<0.001$.
[d]Mean of all weekly values used.
[e]Percent difference compared to control.

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 2000 ppm levels. Tissues designated by (^^) from the 5, 50 and 500 ppm levels were also microscopically examined. The organs designated by (XX) were weighed (all animals and groups), the organ/body weight ratio calculated and the tissues microscopically examined; organs and tissues marked with a (*) were required by the guidelines.

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

Adapted from the original report, p. 16.
X examined microscopically from 0 and 2000 ppm groups
XX weighed and examined microscopically, all animals/groups
^^ microscopic examination from 5, 50 and 500 ppm levels
* specified by the guidelines
[a]with fallopian tubes
[b]with epididymides
[c]female

Organ Weight

Organ weight was determined. An analysis of organ weight/body weight ratio was calculated.

In the male animals, the brain, liver, kidneys and testes showed a decrease in weight compared to the controls at the 2000 ppm dose. The spleen weight showed variation, with higher absolute weight at the 5 and 50 ppm and an increased relative spleen weight; a lower absolute weight was seen at the 2000 ppm dose level (Table 5).

At the 2000 ppm dose level, females animals showed an absolute brain weight decrease (0.42 gm versus 0.45 gm in the control); a relative brain weight increase occurred at 500 (1.45 gm) and 2000 ppm (1.56 gm) dose level versus the control value of 1.36 gm. The liver relative weight was also increased from 5.47 gm in the control to 6.0 gm.

Table 5

Organ Weights (gm) of Male Mice at Necropsy[1,2]

Dose (ppm)	Brain (gm)	Liver (gm)	Spleen (gm)	Kidneys (gm)	Testes (gm)
0	0.44	1.84	0.093	0.59	0.239
5	0.44	1.82	0.113[a]	0.58	0.232
50	0.44	1.83	0.121[b]	0.62	0.235
500	0.43	1.68	0.091	0.56	0.226
2000	0.42[a]	1.44[c]	0.069[a]	0.47[c]	0.206[c]

[1]Adapted from original report, p. 40.
[2]12 animals at each point.
[a]Significantly different from controls, $p<0.05$.
[b]Significantly different from controls, $p<0.01$.
[c]Significantly different from controls, $p<0.001$.

The pathologist noted that only the 5000 ppm dose level showed changes related to treatment with cacodylic acid. "These lesions included soft/mucoid/foamy or hemorrhagic intestinal contents, tympanism (swelling of the abdomen) and congested mucosa. Lesions occurring probably secondary to the cacodylic acid-induced lesions included dehydration, emaciation, reduced or absent fat pads and splenic and thymic atrophy.

Microscopic Pathology

Cecum, Colon and Rectum

A slight diffuse cuboidal to squamous metaplasia of the epithelial columnar absorptive cells was noted at the 2000 ppm dose level in the cecum (92% M, 75% F), colon (58% M, 50% F) and rectum (83% M, 58% F) versus the controls. The author notes that the metaplasia was possibly related to irritation by the test compound (Table 6,7).

Urinary bladder

Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in 100% of the male and female animals at the 500 ppm dose level; at the 2000 ppm dose level, 92% of the males versus 8% in the male control group and 75% of the females versus 0% in the control group showed the lesion (Table 6, 7).

Table 6

Summary of Lesion Incidence in Male Mice in the 13 Week Study[1]

	Males				
Dose (ppm)	0	5	50	500	2000
Number of Organs Examined	12	12	12	12	12
Organ/Lesion:					
Cecum					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	11
%	0	0	0	0	92
Colon					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	7
%	0	0	0	0	58
Rectum					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	10
%	0	0	0	0	83
Urinary Bladder					
Superficial transitional epithelium - vacuolar degeneration	1	0	0	12	11
%	8	0	0	100	92

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[1]Adapted from the original report, p. 53-55.

Table 7

Summary of Lesion Incidence in Female Mice in the 13 Week Study[1]

Dose (ppm)	Females 0	5	50	500	2000
Number of Organs Examined	12	12	12	12	12
Organ/Lesion:					
Cecum					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	9
%	0	0	0	0	75
Colon					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	6
%	0	0	0	0	50
Rectum					
Epithelial columnar absorptive cells - cuboidal to squamous metaplasia, diffuse, slight	0	0	0	0	7
%	0	0	0	0	58
Urinary Bladder					
Superficial transitional epithelium - vacuolar degeneration	0	0	0	12	9
%	0	0	0	100	75

[1]Adapted from the original report, p. 56-58.

DISCUSSION

Females showed a higher intake of the test compound (approximately 25% - 40%) above the corresponding male animals at all dose levels

At the 5000 ppm dose level, females appeared to succumb more quickly to the toxic effects of test compound than the males; at week 2, (1)M and 4F died or was sacrificed *in extremis*, at week 3 (1M, 6F), week 4 (5M, 1F), week 5 (1F), week 6 (2M), week 7 (3 M).

The 5000 ppm dosed animals showed "diarrhea, emaciation, tremors, hunching, hyperthermia and generalized debility."

At the 2000 ppm dose level a decreased rate of body weight gain (30.3% M, 14.8% F) in both sexes occurred together with an increased food consumption (17%) in the females. The efficiency of food conversion decreased (33%) in the male. Water consumption was increased by 28% in females. Males showed decreases in the absolute weight of the brain, liver, spleen, kidneys and testes. Pathological examination of the organs was not remarkable.

At 2000 ppm, both sexes showed slight and diffuse colon, cecum and rectum metaplasia related to the irritative effects of the test compound. Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in 75-92% of the animals of both sexes at the 2000 ppm dose levels versus 8% in the male and 0% in the female control groups.

Effects at the 500 ppm dose level were less severe than at the 2000 ppm dose level. Neither sex showed weight changes (absolute or change of weight gain) but the efficiency of food conversion was decreased (33%) in the male. Water consumption in the female increased by 17% versus the control.

Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in 100% of the animals in both sexes at 500 ppm versus 8% in the male and 0% in the female control groups.

CONCLUSIONS:

Six groups of 12 mice/sex of the B6C3F1 strain were administered cacodylic acid at concentrations admixed in the feed at 0 (Control), 5, 50, 500, 2000 and 5000 ppm for 13 weeks.

Animals at 5000 ppm died or were sacrificed *in extremis* within 8 weeks of the start of the study. No mortality was noted at the lower dose levels.

At 2000 ppm dose level a decrease in the rate of body weight gain of approximately 30% in males and 15% in females occurred together with a 17% increase in food consumption in the females versus the controls. A decrease in the efficiency of food conversion was seen in the males at the 500 and 2000 ppm dose levels. In females, a 17% and 28% increase in water consumption was seen, respectively, at the 500 and 2000 ppm dose levels throughout the study.

In males at 2000 ppm, the brain, liver, kidneys, testes and spleen showed a decrease in organ weights compared to the controls. Females showed an absolute brain weight decrease and a relative brain weight increase at the 500 and 2000 ppm dose level.

Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in both the 500 and 2000 ppm dose levels in both sexes.

MTD: males - <433 mg/kg/day but >102 mg/kg/day (<2000 but >500 ppm)
females - <604 mg/kg/day but >128 mg/kg/day (<2000 but >500 ppm).

NOEL: males - 10 mg/kg/day (50 ppm)
females - 13 mg/kg/day (50 ppm)

LOEL: males (pathology) - 102 mg/kg/day (500 ppm)
females (pathology) - 128 mg/kg/day (500 ppm)

It is the Agency's position that a dose be used in an oncogenicity study that decreases the rate of weight gain by $\geq$10%, the so called Maximum Tolerated Dose (MTD), unless there is justification that this dose cannot be used because of the potential for the effect or lesion to decrease survival on prolonged exposure.

If animals are not dosed at the MTD, the study is considered supplementary. In the mouse oncogenicity study, the MTD was reached in the males but not in the females.

A 13 week study usually defines the toxicity profile (with the exception of carcinogenicity and other specialized toxicities) and can be used to estimate a working MTD for use in a oncogenicity study. In the 13 week study (MRID 423625-01), at the 500 ppm dose level, a decrease in the rate of body weight gain of about 9% occurred in males versus the control. No decrease in the rate of body weight gain was noted on the females. A decrease of less then 10%, however, is deemed by the Agency to be insufficient to define a MTD.

At the 2000 ppm dose level, however, a clear decrease in the rate of body weight gain was noted, i.e., 30% in the males and 15% in the females versus the controls.

The conclusion is, therefore, that a dose level lower than 2000 ppm, but higher than 500 ppm, i.e., 1000 ppm should have been chosen for the high dose level in the mouse oncogenicity study to give some insurance that the dose would have met or exceeded the MTD.

The Agency is also of the opinion that the pathology noted namely, vacuolar degeneration of the superficial transitional epithelium in the urinary bladder at 500 and 2000 ppm would not result in decreased survival on prolonged exposures and should have been of secondary importance for MTD dose selection.

The same can be said of the other parameters cited, such as increased water consumption and relative organ weight change at the 500 ppm dose level.

The Agency is also of the opinion that while certain classes of compounds toxicologically tend to have similar actions, the toxicity of the arsenic compounds is so unique that small molecular changes can profoundly affect the toxicity of the test compound; cacodylic acid, therefore, cannot be compared to other arsenic compounds.

The Agency is in agreement and accepts the rebuttal concerning the exclusion of certain individual organ weights from the means and the explanation of why some clinical pathology were not reported in the oncogenicity study.

It is suggested that another study with a similar protocol be run using a control and high dose level of approximately 1000 ppm; in

so doing a MTD for both male and female animals would, in all probability, be obtained.

The present mouse oncogenicity study (MRID 419146-01), however, must still be labeled as Core - Supplementary. The study does not satisfy the guideline requirement (83-5) for a carcinogenicity study.

Please contact the Agency if more information is desired or you wish to discuss the protocol.

SUMMARY

MRID 423625-01 Subacute Toxicity - mouse (82-1)
Core: Supplementary - not upgradeable

Six groups of 12 mice/sex of the B6C3F1 strain were administered cacodylic acid at concentrations admixed in the feed at 0 (Control), 5, 50, 500, 2000 and 5000 ppm for 13 weeks.

Animals at 5000 ppm died or were sacrificed in extremis within 8 weeks of the start of the study. No mortality was noted at the lower dose levels.

At 2000 ppm dose level a decrease in the rate of body weight gain of approximately 30% in males and 15% in females occurred together with a 17% increase in food consumption in the females versus the controls. A decrease in the efficiency of food conversion was seen in the males at the 500 and 2000 ppm dose levels. In females, a 17% and 28% increase in water consumption was seen, respectively, at the 500 and 2000 ppm dose levels throughout the study.

In males at 2000 ppm, the brain, liver, kidneys, testes and spleen showed a decrease in organ weights compared to the controls. Females showed an absolute brain weight decrease and a relative brain weight increase at the 500 and 2000 ppm dose level.

Vacuolar degeneration of the superficial transitional epithelium of the urinary bladder was seen in both the 500 and 2000 ppm dose levels in both sexes.

MTD: males - <433 mg/kg/day but >102 mg/kg/day (<2000 but >500 ppm)
females - <604 mg/kg/day but >128 mg/kg/day (<2000 but >500 ppm).

NOEL: males - 10 mg/kg/day (50 ppm)
females - 13 mg/kg/day (50 ppm)

LOEL: males (pathology) - 102 mg/kg/day (500 ppm)
females (pathology) - 128 mg/kg/day (500 ppm)