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

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12/6/1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: CACODYLIC ACID - A Developmental Toxicity Study in Rats - (§83-3A)

DP Barcode: D196683 **Case:** 801418 **Caswell No.:** 133
Submission: S453235 **PC Code:** 012501
MRID No.: 406257-01 **Identification No.:** 012501-042519
Action: 627 Generic Data Submission

FROM: Alan C. Levy, Toxicologist Alan C. Levy Dec. 3, 1993
Review Section IV, Toxicology Branch II
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THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 12/03/93
Review Section IV, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 12/6/93
Toxicology Branch II, Health Effects Division (H7509C)

REQUEST: Review a developmental toxicity study in rats with Cacodylic acid.

Registrant: Pamol Ltd., Tel Aviv, Israel

EXECUTIVE SUMMARY:

Developmental toxicity - rat: Core Grade **Minimum** (Pamol Ltd., 1988)

Groups of pregnant Charles River Sqrague-Dawley rats (22/dose) were administered Cacodylic acid via gavage at dose levels of 0, 4, 12 and 36 mg/kg/day during gestation days 6 through 15. Maternal toxicity was observed at the highest dose tested and was as follows: lower body weights, weight gains and food consumption; lower gravid uterine weights (smaller fetuses). Developmental toxicity was observed at the highest dose tested and was as follows: lower fetal body weights, shorter crown-rump length, the suggestion of diaphragmatic hernia and delayed/lack of ossification of numerous bones.

Based on these effects, the NOEL and LEL for maternal toxicity are 12 and 36 mg/kg/day, respectively, and the NOEL and LEL for developmental toxicity are 12 and 36 mg/kg/day, respectively.

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy
Section IV, Tox. Branch II (H7509C) December 3, 1993

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 12/03/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

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

TEST MATERIAL: CACODYLIC ACID; Hydroxydimethylarsine oxide

SYNONYM: Demethylarsinic acid

MRID No.: 406257-01 **PC Code:** 012501 **Caswell No.:** 133

STUDY NUMBER (Project No.): PAL/017/CAC

SPONSOR: Pamol Ltd., Tel Aviv, Israel

TESTING FACILITY: Life Sciences Research Israel LTD
Ness Ziona 70 451, Israel

TITLE OF REPORT: Cacodylic Acid, Teratogenicity Study in the Rat

AUTHORS: N. Gal and Y. Rubin

REPORT ISSUED: April 18, 1988

EXECUTIVE SUMMARY:

Developmental toxicity - rat: Core Grade **Minimum** (Pamol Ltd., 1988)

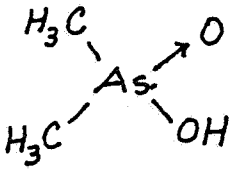
Groups of pregnant Charles River Sprague-Dawley rats (22/dose) were administered Cacodylic acid via gavage at dose levels of 0, 4, 12 and 36 mg/kg/day during gestation days 6 through 15. Maternal toxicity was observed at the highest dose tested and was as follows: lower body weights, weight gains and food consumption; lower gravid uterine weights (smaller fetuses). Developmental toxicity was observed at the highest dose tested and was as follows: lower fetal body weights, shorter crown-rump length, the suggestion of diaphragmatic hernia and delayed/lack of ossification of numerous bones.

Based on these effects, the NOEL and LEL for maternal toxicity are 12 and 36 mg/kg/day, respectively, and the NOEL and LEL for developmental toxicity are 12 and 36 mg/kg/day, respectively.

I. TEST ARTICLE

Name: Cacodylic acid; Dimethylarsinic acid; Hydroxydimethylarsine oxide

Formula:



Purity: 99.8%
Lot No. (Batch No.): 1007
Appearance: white crystalline powder

II. MATERIALS, METHODS AND RESULTS

A. Statistical Analyses

MATERNAL

Student's t test: food consumption, body weight and body weight change
Fisher exact test: clinical signs and necropsy findings

FETAL

Mann-Whitney U-test: pre- and post-implantation loss and individual litter proportions
Student's t test: Freeman-Tukey transformed data, weight, length and placental weight
Chi-square or Fisher exact test: number of affected fetuses and number of affected litters

B. Regulatory Compliance

A Good Laboratory Practice Compliance statement, Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

A statement of "no claim of confidentiality" was included in the Report.

There was no statement by the Registrant applying the criteria of 4 CFR 158.34 regarding flagging studies for potential adverse effects.

C. Dose Selection

The Report indicated that the doses chosen for this study (0, 4, 12 and 36 mg/kg/day) were based on the results of a preliminary developmental toxicity study (LSRI Report No. PAL/016/CAC). The results of this study were not included in the Guideline study.

D. Test Article and Dosing Solutions

Cacodylic acid dosing solutions (vehicle of double distilled water) were formulated up to 14 days prior to administration. Doses of 0 (distilled water), 4, 12 and 36 mg/kg/day at a volume of 10 ml/kg were administered by gavage daily to rats on gestation days 6 through 15. The administered dose was based on the rat's body weight on the dosing day.

E. Test Article Stability and Concentration (Table 1)

Table 1

STABILITY AND CONCENTRATION OF CACODYLIC ACID DOSING SOLUTIONS IN A RAT DEVELOPMENTAL TOXICITY STUDY

Desired		Concentrations found (mg/ml)	
Dose (mg/kg)	Conc. (mg/ml)	Time 0	15 Days Later
4	0.4	0.40(100)	0.375(94)
12	1.2	1.19(99)	1.10(92)
36	3.6	3.52(98)	3.33(93)

NOTE: all concentrations found represent the mean of 2 assays
() = % of desired concentration
Data extracted from Report page 59.

Stability and concentration analytical data were considered to be within acceptable limits.

F. Animals and Mating

Sexually mature Charles River Breeding Laboratories (U.K.) Ltd. Sqrague-Dawley CD rats were used for this study. Two groups of females were 48-54 days old (160-175 g) and 55-65 days old (190-195 g). There was a 6 day period of acclimation prior to pairing with males (same strain and source). Females were individually housed except during mating. Food and water

were available ad libitum. Room temperature and humidity were targeted for 21 ± 2°C and 55 ± 15%, respectively. The Report did not mention the light/dark cycle.

One female was paired with one male. When copulation plugs were found, a vaginal smear was made for the estimation of the amount of sperm in the vaginal tract. Mated females were assigned to dose groups in the order of mating. No male was mated with a disproportionate number of females in any group and group mean body weights were similar. The day the plug and sperm were observed was designated as gestation day 0.

G. Clinical Observations and Survival

Animals were observed daily for mortality and moribundity as well as clinical signs.

All rats survived and there were no clinical signs attributed to test article administration.

H. Body Weights

Body weights were recorded on gestation days 0, 3, 6-15, 17 and 20. Weight changes were calculated for days 0-6, 6-15, 15-20 and 0-20. See Table 2.

Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A DEVELOPMENTAL TOXICITY STUDY IN RATS WITH CACODYLIC ACID

	Gestation Days										
Dose	Body Weights (g)							Body Weight gain (g)			
mg/kg	0	6	9	12	15	20	C	0-6	6-15	15-20	0-20
0	227	256	268	284	303	377	298	29	47	74	150
4	229	258	270	285	302	375	297	29	44	72	145
12	227	255	266	284	303	376	298	28	48	73	149
36	229	257	268	279	290 **	355 ***	290	28	33 ***	65**	126 ***

Number of Rats/group = 22
C = Terminal body weight minus gravid uterine weight.
Statistical Significance: ** = $p<0.01$; *** = $p<0.001$
Data extracted from Report Table 3, pages 27 and 28.

At 36 mg/kg/day, body weights were significantly ($p<0.01$ or 0.001) below control means on weighing days 15, 17 (in Report, not shown in Table 2) and 20. Body weight gains for the 36 mg/kg/day group were significantly ($p<0.01$ or 0.001) less than controls for interval days 6-15 (dosing), 15-20 (post-dosing) and for 0-20 (entire gestation period). There were no apparent affects on group mean body weights or weight gains at the 4 or 12 mg/kg/day dose groups. The terminal body weights minus gravid uterine weights were similar for all groups: 298 ± 15 (S.D.), 297 ± 16, 298 ± 17 and 290 ± 14 at 0, 4, 12 and 36 mg/kg/day, respectively. These data indicate that the lower body weights and weight gains in the 36 mg/kg/day group were primarily due to the weight of gravid uteri. [Report Table 4, page 29, showed gravid uterine weights of 80, 77, 79 and 65 ($p<0.001$) g for the 0, 4, 12 and 36 mg/kg/day groups, respectively.]

I. Food Consumption

Individual food consumption was recorded during gestation days 1-3, 4-6, 7-9, 10-13, 14-16 and 17-20. See Table 3.

Table 3

GROUP MEAN FOOD CONSUMPTION IN A DEVELOPMENTAL TOXICITY STUDY IN RATS WITH CACODYLIC ACID

Dose mg/kg	Gestation Days (g/rat/day)					
	1-3	4-6	7-9	10-13	14-16	17-20
0	27	26	26	26	27	30
4	27	26	26	26	27	30
12	26	26	26	26	27	30
36	27	26	25	23***	22***	30

Number of rats/group = 22
Statistical significance: *** = $p<0.001$
Data extracted from Report Table 2, page 26.

Food consumption was significantly ($p<0.001$) lower in the 36 mg/kg/day group compared with the control during gestation days 10-16. The values for the 4 and 12 mg/kg/day groups were similar to controls.

J. Cesarean Section Observations

All females were sacrificed by carbon dioxide asphyxiation on day 20 of gestation. The uterus and ovaries were removed and the number of corpora lutea recorded. The uterus was weighed and the following were recorded: live/dead fetuses, early/late resorption sites, individual placental weights, fetal weights, fetal crown-rump length and fetal sex. Table 4.

Table 4

CESAREAN SECTION DATA FROM A DEVELOPMENTAL TOXICITY STUDY IN RATS WITH CACODYLIC ACID

Parameter mg/kg/day =	0	4	12	36
Females mated (number)	22	22	22	22
Number pregnant (percent)	22(100)	22(100)	22(100)	22(100)
Litters with viable fetuses	22	22	22	22
Maternal mortality	0	0	0	0
Abortions	0	0	0	0
Corpora lutea (mean)	17.6	16.6	16.5	13.6**
Weight of gravid uterus (g)	80.0	77.2	78.8	64.5***
Pre-implantation loss (mean percent)	6.7	5.8	4.8**	4.6***
Post-implantation loss (mean percent)	6.1	8.6*	6.8	8.6*
Viable fetuses (mean)	15.2	14.4	14.8	13.6*
Resorptions (mean percent)	1.1	1.1	1.0	1.4
Mean body weight viable fetuses (g)	3.4	3.4	3.4	2.9***
Mean crown-rump length (mm)	35.5	36.0	36.0	33.7***
Number of fetuses weighed (examined)	335	316	326	300
Small fetuses (<3.0 g or >0.5 g below litter mean) - mean(%)	37(11)	30(10)	24(7)	184(61) ***

Statistical Significance: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$
Data extracted from Report Tables 4 and 5, pages 29 and 30.

Statistically significant "negative" effects were primarily noted in the 36 mg/kg/day group. Although the mean number of corpora lutea were less than the control (13.6 versus 17.6, $p<0.01$), this is not considered an effect of test article administration as the number of corpora lutea was established prior to the start of dosing.

Gravid uterine weight at 36 mg/kg/day was less than control (64.5 versus 80.0 g, $p<0.001$).

Post-implantation loss was greater ($p<0.05$) in the 4 and 36 mg/kg/day groups than in the control. The lower dose value was not likely to have been due to Cacodylic acid administration as there was little or no increase at the mid-dose (12 mg/kg/day). The 8.6% loss at 36 mg/kg/day was primarily due to rat No. 85 which had 13 corpora lutea, a gravid uterine weight of 13 g, 2 live fetuses and 11 late resorptions. If rat No. 85 data are excluded, the group mean percent post-implantation loss is 6.5 ± 2.2% (if included, 8.6 ± 5.5%).

The group mean number of viable fetuses in the 36 mg/kg/day group was 13.6 compared with a control value of 15.2 ($p<0.05$). However, the mean number of corpora lutea in the two groups were 13.6 and 17.6.

Fetal size (weight and crown-rump length) was below the control values ($p<0.001$) in the 36 mg/kg/day group, and the number of fetuses that weighed <3.0 g or were >0.5 g below the litter mean, were 184/300 (61%) versus control values of 37/335(11%), $p<0.001$.

K. Fetal Observations

All fetuses were subjected to examination. About 1/2 of the fetuses from each litter were dissected and examined for thoracic and abdominal abnormalities. These fetuses, after evisceration, were processed for skeletal staining with Alizarin Red S. The rest of the fetuses from each litter were fixed in Bouin's fluid and were examined ("free-hand sections") for visceral observations.

1. EXTERNAL AND VISCERAL

Diaphragmatic hernia was reported in the 36 mg/kg/day group: at necropsy, one fetus from female No. 71 and 2 fetuses from female No. 80; from "free-hand section", one fetus from female No. 80 and one fetus from female No. 86. Therefore, there were a total of 5/300 fetuses (1.7%) from 3/22 litters (13.6%) [$p<0.05$] with this observation compared to none in the 0, 4 or 12 mg/kg/day groups. No historical control data were included in the report.

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2. SKELETAL - Table 5

Table 5

STATISTICALLY SIGNIFICANT SKELETAL OBSERVATIONS IN A DEVELOPMENTAL TOXICITY STUDY IN RATS WITH CACODYLIC ACID

	Number (%) of Fetuses				Litter Distrib. No.(%b)			
Observation	0a	4	12	36	0a	4	12	36
Number examined	168	159	164	150	22	22	22	22
Anterior fontanelle - small	1 (1)	6 (4)*	7 (4)*	8 (5)*	1 (1)	4 (4)	5 (5)	5 (5)
Supraoccipital-reduced or incomplete ossification	24 (14)	28 (18)	18 (11)	65 (43) ***	11 (14)	11 (17)	9 (10)	17 (43) **
No. examined (hyoid)	141	128	148	145	22	22	22	22
Hyoid-reduced or incomplete ossification	1 (1)	6 (5) *	1 (1)	28 (19) ***	1 (1)	5 (7)	1 (1)	11 (21) ***
One or two thoracic vetebral centra incompletely fused	42 (25)	46 (29)	59 (36)	58 (39)	17 (26)	19 (28)	20 (36)	20 (40) *
Three or more thoracic vertebral centra incompletely fused	8 (5)	4 (3)	2 (1)	18 (12) *	4 (3)	4 (5)	2 (1)	8 (10)
One or two thoracic vertebral centra bipartite	3 (2)	2 (1)	3 (2)	9 (6) *	2 (2)	2 (2)	3 (2)	7 (6)
13th rib rudimentary	1 (1)	2 (1)	2 (1)	13 (9) ***	1 (1)	2 (2)	2 (1)	8 (8) **
One or more sternebrae 1-4 unossified	8 (5)	3 (2)	2 (1)	24 (16) **	4 (6)	3 (2)	2 (1)	11 (14) *
Irregular ossification of 1 or more of sternebrae 1-4	6 (4)	5 (3)	6 (4)	66 (44) ***	4 (4)	3 (3)	5 (4)	15 (43) ***
Metacarpus V unossified bilaterally	102 (61)	96 (60)	103 (62)	134 (89) ***	20 (60)	21 (59)	21 (62)	22 (89) ***
Metatarsus V unossified bilaterally	0 (0)	1 (1)	0 (0)	6 (4) *	0 (0)	1 (1)	0 (0)	4 (4)
Pubic bone-reduced or incomplete ossifica. or unossified	4 (2)	15 (9) **	5 (3)	14 (9) **	3 (2)	5 (9)	3 (3)	6 (8)

a = mg/kg/day; b = % of mean of individual litter proportions
Statistical Significance: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$
Data extracted from Report Tables 9 and 10, pages 39-50.

Essentially, all statistically significant ($p<0.05$, 0.01 or 0.001) skeletal differences were observed in the 36 mg/kg/day group compared with the control group. As noted in Table 5, with the exception of an increase in 13th rudimentary ribs, all of the findings were related to a decrease in fetal growth rate (reduced or lack of ossification). These skeletal observations coincide with small fetuses.

III. DISCUSSION

Test article stability and concentration were considered to be within acceptable ranges.

There were no maternal deaths. Cacodylic acid did not appear to cause any clinical signs which were different from those seen in control rats.

At 36 mg/kg/day, as compared with controls, there were statistically significant lower body weights at gestation days 15 and 20 as well as decreased weight gains during days 6-15, 15-20 and 0-20. This was due to lighter gravid uteri (smaller litter size and smaller fetuses), as the final group mean body weights minus gravid uterine weights for the control and treated groups were similar. Concomitantly, there was less food consumed (g/rat/day) during gestation days 10-16.

There was a decrease in the group mean number of corpora lutea at 36 mg/kg/day. As dosing began on gestation day 6, this finding is not considered to be an effect of Cacodylic acid treatment.

The significantly lower group mean number of viable fetuses at 36 mg/kg/day is not considered to be a test article effect as the mean number of viable fetuses was the same as the mean number of corpora lutea.

Cesarean section effects of 36 mg/kg/day were decreased gravid uterine weights and smaller fetuses (weights and crown-rump length).

A greater post-implantation loss shown at 36 mg/kg/day, was most likely due to one rat which had 13 corpora lutea with 2 live fetuses and 11 late resorption sites. Even though the 4 mg/kg/day group had a significantly greater percent of post-implantation loss, this was not observed at 12 mg/kg/day. Therefore, it is only questionable that the test article had an effect on this parameter.

Three 36 mg/kg/day litters had a total of 5 fetuses with diaphragmatic hernias (observed either grossly or by "free-hand section"). This finding was not reported in any other groups. Both the fetal (13.6%) and the litter (1.7%) incidences exceeded the concurrent control (0%) and the spontaneous incidences (mean: fetal = 2/3617, 0.06% and litter = 2/517, 0.39%; range: fetal = 0-0.9% and litter = 0-5.6%) observed in studies conducted with Charles River CD

rats between 1984-1987 (source: Charles River, 1988). It is therefore suggested that 36 mg/kg/day of Cacodylic acid may have produced this abnormality.

Fetal skeletal findings were categorized as a result of delayed growth of the fetuses. There were decreases in or lack of ossification of numerous bones. In addition, there were more fetuses and litters with the observation of 13th rudimentary ribs. The presence of the 13th rib is not considered to be of a serious nature. No historical control data were provided.

IV. EXECUTIVE SUMMARY:

Developmental toxicity - rat: Core Grade **Minimum** (Pamol Ltd., 1988)

Groups of pregnant Charles River Sprague-Dawley rats (22/dose) were administered Cacodylic acid via gavage at dose levels of 0, 4, 12 and 36 mg/kg/day during gestation days 6 through 15. Maternal toxicity was observed at the highest dose tested and was as follows: lower body weights, weight gains and food consumption; lower gravid uterine weights (smaller fetuses). Developmental toxicity was observed at the highest dose tested and was as follows: lower fetal body weights, shorter crown-rump length, the suggestion of diaphragmatic hernia and delayed/lack of ossification of numerous bones.

Based on these effects, the NOEL and LEL for maternal toxicity are 12 and 36 mg/kg/day, respectively, and the NOEL and LEL for developmental toxicity are 12 and 36 mg/kg/day, respectively.

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