SECTION HEAD

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

OCT 15 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: CACODYLIC ACID: Review of Chronic Toxicity Study - dog [Guideline 83-1]

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 10/5/93
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Tom Myers, PM (51)
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THRU : Jess Rowland, M.S., Acting Section Head Jess Rowland 10/6/93
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and

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Task Identifications: Submission: S411281 DP Barcode: D174260
P.C. Code: 012501 Caswell No.: 133
HED Project No. 2-1489

ACTION REQUESTED: Review of Chronic Toxicity Study in Dogs with Cacodylic Acid **[MRID No: 414909-01]** for reregistration.

RESPONSE: A Data Evaluation Report [DER] for the above referenced study is attached. A summary is provided below.

Cacodylic acid (99.8%) was administered as a single oral dose by capsule to 4 groups of 5 purebred beagle dogs per sex at dose levels of 0 (Control), 6.5, 16 and 40 mg/kg/day 6 days a week for 52 weeks.

A dose related increase in the diarrhea incidence occurred in both sexes while an increase in the incidence of vomiting occurred in females. Vomiting was increased at the high dose in the male.

Decreases in body weight gain occurred in the high dose males while body weight and body weight gain decreased in high dose females.

High dose level males showed decreases in total protein and albumin at 12, 25, 39 and 51 weeks while high dose females showed decreases in albumin on week 39 and total protein on week 51.

Decreases in HCT, HgB and RBC occurred in the high dose males at weeks 12, 25 (dose related) and 51.

Based on the results of this study, the following NOEL and LOEL are established: NOEL = 16 mg/kg/day; LOEL = 40 mg/kg/day. The LOEL is based on clinical signs, decrease body weight and body weight gain and alterations in clinical chemistry and hematology.

CORE CLASSIFICATION: Minimum

This study satisfies the data requirements (83-1) for a Chronic Toxicity Study and is acceptable for regulatory purposes.

Reviewed by Steven L. Malish, Ph.D., Toxicologist S. L. Malish 10/5/93
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Jess Rowland, M.S., Acting Section Head
Tox. Branch II, Section IV (H7509C) Jess Rowland 10/6/93.

Data Evaluation Report

STUDY TYPE: 83-1 Chronic Toxicity Study - Dog

MRID NO: 414909-01

TEST MATERIAL: Cacodylic Acid

SYNONYMS: Dimethylarsinic acid, Cacodylic acid

SPONSOR: Luxembourg Industries (Pamol) Ltd.
27 Hamered St. P.O. 13
Tel-Aviv 61000, Israel

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

LAB STUDY NO.: PAL/012/CAC

TITLE OF REPORT: Cacodylic Acid
52-Week Oral Toxicity Study in Beagle Dogs

AUTHORS: G. Zomber, A. Nyska, T. Waner, M. Pirak,
S. Crown

REPORT ISSUED: November 21, 1989

SUMMARY:

Cacodylic acid (99.8%) was administered as a single oral dose by capsule to 4 groups of 5 purebred beagle dogs per sex at dose levels of 0 (Control), 6.5, 16 and 40 mg/kg/day 6 days a week for 52 weeks.

A dose related increase in the diarrhea incidence occurred in both sexes while an increase in the incidence of vomiting occurred in females. Vomiting was increased at the high dose in the male.

Decreases in body weight gain occurred in the high dose males while body weight and body weight gain decreased in high dose females.

High dose level males showed decreases in total protein and albumin at 12, 25, 39 and 51 weeks while high dose females showed decreases in albumin on week 39 and total protein on week 51.

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Decreases in HCT, HgB and RBC occurred in the high dose males at weeks 12, 25 (dose related) and 51.

Based on the results of this study, the following NOEL and LOEL are established: NOEL = 16 mg/kg/day; LOEL = 40 mg/kg/day. The LOEL is based on clinical signs, decrease body weight and body weight gain and alterations in clinical chemistry and hematology.

CORE CLASSIFICATION: Minimum

This study satisfies the data requirements (83-1) for a Chronic Toxicity Study and is acceptable for regulatory purposes.

A. MATERIALS:

1. Test Compound

Common name:	cacodylic acid
Batch No:	1007
Chemical name:	hydroxydimethylarsine oxide
CAS:	75-60-5
Purity:	99.8% w/w (technical)
Description:	white crystalline solid
Stability:	stable under laboratory storage conditions

2. Test Animals

Species:	dog
Strain:	pure bred Beagle
Age:	approximately 20 weeks of age on arrival
Weight:	mean weight males 9.93±1.05 kg; females 9.22±1.19 kg on first weighing 2 days after arrival
Source:	Central Institute for the Breeding of Laboratory Animals, Harlan/CPB Zeist, The Netherlands

B. STUDY DESIGN:

Animal Assignments

Animals were individually housed individually and acclimatized for approximately 32 days. Dogs were assigned to their respective treatment groups using a body weight allocation method.

Forty (40) animals per sex were randomly assigned to four (4) groups and administered 0 mg/kg/day, (Control, Group 1), 6.5 mg/kg/day (Group 2), 16 (group 3) and 40 mg/kg/day (Group 4) orally by capsule once a day (Table 1).

Table 1

Animal Test Group Assignments[1]

Group	Treatment	Dose (mg/kg/day)	Animals on Test M/F
1	Control	0.0	5/5
2	Cacodylic acid	6.5	5/5
3	Cacodylic acid	16.0	5/5
4	Cacodylic acid	40.0	5/5

Test Material Dose Preparation and Administration

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.

All dogs were dosed orally by capsule, once a day, 6 days a week, except Saturday.

Diet

Animals received a daily diet of a complete pelleted dog diet (Dog Breeding Maintenance Diet, No. 4134, Altromin Spezialfutterwerke. Lage, West Germany).

Water Supply

Drinking water was supplied to each cage ad libitum via an automatic "Luxit" valve system.

Statistics

The following data were subjected to statistical analysis:

body temperature, body weight, food consumption, organ weights, blood chemistry, hematology and urine parameters.

The initial stage of analysis was performed using the Bartlett's test for homogeneity of variance. Where variances were homogenous at the 1% level, the data were subjected to a one-way parametric ANOVA. If the ANOVA showed a significant difference between the groups, then Dunnett's t-test was employed to test for differences between the control and treatment groups.

Where the variances were non-homogenous, then the Kruskal-Wallis non-parametric ANOVA was employed instead of the parametric ANOVA and the Dunn test was substituted for the Dunnett's t-test.

Absolute organ weight and organ weight using body weight as a covariant were analyzed.

Physical Examination

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

Teeth and gums, mucous membrane 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.

The physical examination in all examination periods in all dose groups was considered to be unremarkable.

Regulatory Compliance

A quality assurance statement and a statement of compliance with Good Laboratory Practice Standards were signed and dated.
A statement of no data confidentiality claims was signed and dated.
A flagging statement was included in the report - "This study neither meets nor exceeds any of the applicable criteria."

C. METHODS AND RESULTS:

Observations

Dogs were inspected 3 times daily throughout the work week and on Sunday. No dosing occurred on Saturday; dogs were inspected twice daily. All signs in the treated groups were observed starting during the first week of dosing. Salivation was observed prior to the daily dosing. Diarrhea and vomiting were observed most often at the afternoon check, to a lesser extent at the morning check and least frequently at the pre-dosing examination. The frequency of the clinical signs was lower on the morning [Sunday] following the day the animals were not dosed [Saturday]. The incidence of clinical signs were seen in Tables 2 and 3.

The mean incidence of salivation and diarrhea in both sexes and the vomiting incidence in the females increased in a dose related manner in the treated animals vs. the respective controls.

In the males, an increase in vomiting was observed at all treated animals vs. the control but was not dose related. At all dose levels, the vomiting incidence generally diminished as dosing progressed. Vomiting of the dosing capsule was rarely seen.

The diarrhea incidence in the male at the mid-dose and the female at the low dose was reduced during weeks 27 to 55 vs. weeks 1 to 26.

In the male at the mid and high doses, the incidence of salivation peaked during weeks 14-26; throughout the study, the salivation incidence decreased slightly at the mid-dose but remained constant at the high dose. In the females, the salivation incidence at the low dose peaked at 27-39 weeks, while the high dose incidence peaked at 14-26 weeks; thereafter, the incidence of salivation at both doses remained constant until the end of the study. The mid-dose showed the highest incidence at the 40-55 week period.

Table 2

Incidence of Clinical Signs (%) Throughout the Study in Male Beagle Dogs Treated with Cacodylic Acid[1,2]

Sign	Weeks on Test 1-13 (%)	14-26 (%)	27-39 (%)	40-55 (%)	Mean[3] (%)
Salivation					
0.0 mg/kg/day	0.0	0.0	0.3	0.0	0.1
6.5	0.3	0.3	0.8	0.7	0.5
16.0	9.2	30.0	21.8	22.3	18.6
40.0	72.8	93.7	89.2	90.4	86.5
Vomiting					
0.0 mg/kg/day	1.6	0.6	1.1	0.2	0.9
6.5	8.5	7.8	3.0	2.0	5.3
16.0	6.0	1.2	1.1	0.6	2.2
40.0	12.5	14.1	6.4	3.9	9.2
Diarrhea					
0.0 mg/kg/day	4.0	11.3	2.0	3.3	5.2
6.5	5.9	10.3	3.9	4.0	6.0
16.0	23.2	28.9	6.5	4.9	15.9
40.0	38.5	48.4	30.2	56.6	43.4

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[1]Adapted from original report, Vol. I, p. 39 thru 47.
[2]Incidence of clinical signs per total number of observations.
[3]Mean of the signs during the weekly observation periods.

Table 3

Incidence of Clinical Signs (%) Throughout the Study in Female Beagle Dogs Treated with Cacodylic Acid[1,2,3]

Sign	Weeks on Test 1-13 (%)	14-26 (%)	27-39 (%)	40-55 (%)	Mean[4] (%)
Salivation					
0.0 mg/kg/day	0.0	0.0	0.0	0.0	0.0
6.5	2.1	4.4	22.3	23.0	13.0
16.0	10.5	16.9	7.9	36.2	17.9
40.0	78.9	100.4	101.0	100.4	95.2
Vomiting					
0.0 mg/kg/day	2.3	1.6	1.3	0.6	1.5
6.5	14.3	14.7	5.9	2.5	9.4
16.0	19.8	14.0	8.5	6.8	12.3
40.0	29.0	23.1	14.6	10.8	19.4
Diarrhea					
0.0 mg/kg/day	7.2	4.6	2.0	2.0	4.0
6.5	11.2	13.8	6.8	5.9	9.4
16.0	10.4	22.1	14.0	11.2	14.4
40.0	37.9	54.2	33.9	50.2	44.1

[1]Adapted from original report, Vol. I, p. 39 to 47.
[2]Incidence of clinical signs per total number of observations.
[3]Values may be greater than 100% due to the fact that the same animal may have been counted at the 3 observation periods per day.
[4]Mean of the signs during the weekly observation periods.

Food Consumption

Food was given as 2 meals of 200 gms each in the morning with the daily dose and in the afternoon. The weight of the food not consumed was measured each day. The procedure was performed daily during the final 2 weeks of acclimation and throughout the treatment period. From these data, the mean daily intake for each animal was calculated on a weekly basis.

Food consumption in all dose groups was considered to be unremarkable.

Body Weight

Each animal was weighed weekly throughout the acclimation and study periods.

At termination, when compared to the controls, the mean body weights were lower in males only at the high-dose [-6%] whereas

females exhibited lower body weights at all dose levels -7%, -2% and -15% at low, mid and high-doses, respectively (Table 4). At termination, mean body weight gain was -29% in males and -42% in females when compared to the respective controls. The decrease in body weight gain noted in females in the lower dose [-30%] was not considered to be toxicologically significant due to a lack of a dose response [-9% at the mid-dose] and the lack of other treatment-related effects at the low-dose (Table 4).

Table 4

Mean Body Weights (kg) in Dogs Treated for 52 Weeks with Cacodylic Acid[1]

	Group/Sex							
Week	1M	2M	3M	4M	1F	2F	3F	4F
0	11.2	11.4	11.6	11.3	9.5	9.8	9.7	9.3
5	12.4	12.3	12.6	12.0	10.6	10.7	10.6	10.2
10	13.3	13.1	13.2	12.4	11.4	11.1	11.3	10.7
25	14.0	14.2	14.3	13.0	12.7	11.7	12.5	11.6
40	13.8	14.4	14.2	12.9	12.9	11.7	12.6	11.6
53	14.7	15.3	15.2	13.8	13.8	12.8	13.6	11.8
Diff[2]	3.5	3.9	3.6	2.5	4.3	3.0	3.9	2.5
% Change[3]	--	11	3	-29	--	-30	-9	-42
% Absolute[4]	--	4	3	-6	--	-7	-2	-15

[1]Adapted from the original report Vol. I, p. 62 to 73.
[2]Difference (kg) from week 0 to week 53.
[3]Percent difference (Diff) in the rate of body weight gain from the week 0 to week 53 compared to the corresponding control. Statistical evaluation not performed.
[4]Percent difference in absolute body weight compared to the corresponding control at week 53. No statistically significant differences were reported.

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 the local anesthetic 0.5% Tropicamide®.

No treatment-related ophthalmological effects were noted.

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:

Cranial nerve reflexes, segmental reflexes, postural reactions, and general observations.

No treatment related effects were seen in dogs at any dose level.

Clinical Pathology

Blood samples were withdrawn from the jugular vein of each dog, 20-24 hours after dosing and after overnight starvation. The following hematology and clinical chemistry examinations were carried out before commencement of treatment and after 12, 29, 39 and 50 weeks of treatment.

Hematology parameters determined in the study were designated by an (X). The parameters marked with an (*) were designated in the latest guideline.

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (Hb)*
X Erythrocyte count (RBC)*
X Leukocyte count*
X Leucocyte differential count*
X Prothrombin time*
X Partial prothrombin time
X Platelet count*
X Mean corpuscular volume
X Mean cell hemoglobin concentration [MCHC]
X Erythrocyte sedimentation rate

A transient dose related decline in the hematocrit (HCT), hemoglobin (HgB) and red blood cell (RBC) count was noted in the males after 25 weeks of treatment reaching statistical significance at the high dose; the RBC count at the mid-dose also showed statistical significance. No changes were noted in mean cell hemoglobin concentration or in the mean cell volume in these groups. Decreases in HCT, HgB and RBC count were also seen in males at the high dose during weeks 12 and 51; the decreased did not reach statistical significance at these intervals except for the RBC count at week 51 (Table 5). Hematology parameters in the females were not affected at any dose level or time period.

Sporadic effects were occasionally noted in other hematology parameters but were not considered to be of any toxicological significance.

Table 5

Hematological Changes Observed in Male Dogs

	Week 12			Week 25			Week 51		
Dose (mg/kg /day)	HCT (%)	HgB (gm %)	RBC (10^6/ ul)	HCT (%)	HgB (gm %)	RBC (10^6/ ul	HCT (%)	HgB (gm %)	RBC (10^6/ ul)
0	46.4	16.0	6.77	46.3	16.8	6.98	50.7	16.6	7.61
6.5	43.6	14.9	6.37	43.5	15.8	6.44	47.2	15.3	7.00
16.0	45.4	15.9	6.81	42.8	15.5	6.33*	42.9	16.0	7.20
40.0	42.6	14.7	6.38	41.7*	15.1**	6.21**	46.6	15.4	6.88*

*p<0.05
**p<0.01

b. Clinical Chemistry

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

X Blood creatinine
X Blood urea nitrogen*
X Cholesterol*
X Glucose (fasting)*
X Total serum protein*
- Triglycerides*
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Albumin (by electrophoresis)*
X Globulin (by electrophoresis)
X Gamma glutamyl transpeptidase
X Creatinine phosphokinase (CPK)
X Alkaline phosphatase
X Bilirubin (total)
X Total lactic dehydrogenase
X Chloride *
X Potassium*
X Sodium*
X Calcium*
X Phosphorous*

A decrease in total plasma protein concentration and concomitant decline in the level of albumin were detected in the high dose males throughout the study (Table 6).

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At week 39, high dose level females showed a decreased albumin concentration of 26.9 gm/l compared to a control value of 33.3 gm/l. On week 51, the same group showed a decrease in total protein of 57.3 gm/l versus 67.1 gm/l in the control group (Table 6).

Calcium levels were decreased on Week 25 in the high dose level males from 2.46 micromoles per liter (uM/l), $p<0.05$) to 2.70 uM/l in the control group. Similarly on week 39 the high dose level males were noted to have a depressed value of 2.43 uM/l ($p<0.05$) compared to 2.61 uM/l in the control group. In the high dose level females, a decreased value of 2.50 uM/l ($p<0.05$) compared to 2.73 uM/l was seen in the control group. These spurious values were not considered to be of any toxicological significance. Results were considered to be unremarkable at the other examinations and time periods.

Effects noted in other parameters were not considered to be of any toxicological importance.

Table 6

Total Protein/Albumin Concentration (gm/l) in Male Beagle Dogs[1]

	Group/Sex			
Week	1M	2M	3M	4M
0	62.0/30.1	62.6/29.8	60.8/31.6	63.5/31.8**
12	57.8/29.8	56.5/28.9	68.1/29.4	54.0*/24.7***
25	65.1/33.5	63.8/30.5	63.8/59.3	59.3*/26.5***
39	66.0/34.3	67.6/31.5	66.5/32.3	57.7***/25.06***
51	66.0/34.0	65.3/32.1	64.0/33.6	57.8***/26.7***

[1]Adapted from original report, Vol. I, p. 96 to 110.
*Significantly different from controls, $p<0.05$.
**Significantly different from controls, $p<0.01$.
***Significantly different from controls, $p<0.001$.

c. Urinalysis

Urine samples were collected before commencement of treatment and after 12, 29, 39 and 50 weeks of treatment.

A urine sample was collected from each animal approximately 6 to 22 hours after dosing, under conditions of food and water deprivation.

The following parameters marked with an (X) were examined. Parameters marked with an (*) were required by the guidelines.

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

Urine sediment was examined microscopically for: epithelial cells, polymorphonuclear leukocytes, red blood cells, casts, crystals and other abnormal components.

No effects of biological importance were noted throughout the study.

Sacrifice and Pathology

All animals were administered intravenous sodium pentobarbital anesthesia and sacrificed by rapid exsanguination and necropsied for gross and microscopic pathology during week 53 thru 55 of treatment.

The checked (X) tissue were collected for gross and histological examinations from all animals. The organs designated by (XX) were weighed and the organ and body weight were compared by covariant analysis. Organs and tissues marked with a (*) were required by the guideline.

Digestive	Cardiovas./ Hematology	Neurologic
X tongue	X aorta*	XX brain*
X esophagus*	XX heart*	- peripheral nerve*
X stomach*	X bone marrow*	X spinal cord (3 levels)
X duodenum*	X lymph nodes*	X sciatic nerve
X jejunum*	cervical/	XX pituitary*
X pancreas	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*	XX thyroids*
XX liver*	X urinary bladder	Other
X pancreas*	XX testes(a)*	X bone*
Respiratory	X prostate*	X skeletal muscle*
X trachea*	X seminal ves.	X skin*
X lung*	XX ovaries*	X unusual lesions*
	X uterus*	X salivary gland*
	X vagina	X target organs
		X mammary gland (female)
		X gall bladder*

(a) with epididymides
X examined microscopically
XX weighed and examined microscopically

Gross and microscopic pathology was considered to be unremarkable.

a. Organ Weights

Absolute organ weights were similar in control and treated groups.

When organ weights were analyzed using terminal necropsy body weight as a covariant, a statistically significant increase in the liver weight was detected in the high dose males; low dose males showed an increase in thyroid weight (Table 7).

Table 7

Liver and Thyroid Weight Using Terminal Body Weight as a Covariant in Male Beagle Dogs[1]

	Least Square Means[2]	
Group	Liver	Thyroids
1M	359.7±12.0	1.03±0.08
2M	369.3±12.3	1.31**±0.08
3M	362.2±12.2	0.86±0.08
4M	418.3*±12.7	1.02±0.08

[1]Adapted from original report, Vol., I, p. 123.
[2]gm±S.E.(S.E. = standard error)
*p<0.05
**p<0.01

DISCUSSION

In the high dose level animals, the vomiting and diarrhea were sufficient to cause a decrease in the body weight gain in the males and a decrease in body weight gain and body weight in the females.

Low dose level females showed a 30% decrease in body weight gain when compared to the control. The cause of the lower body weight gain in the low dose dogs is not known at this time since other clinical parameters and microscopic pathology were considered to be within normal limits for these dogs. Mid dose level animals showed a rate of weight gain similar to the control.

Alteration of hemoglobin parameters (HCT, HgB, RBC) were not considered to be treatment related due to the lack of collaborative histopathological changes in the hematopoietic system.

A decrease in total plasma protein concentration and concomitant decline in the level of albumin were detected in the high dose males throughout the study.

High dose level females showed a decreased albumin concentration on Week 39 and 51.

When absolute organ weights were statistically analyzed using body weight as a covariant, liver weight was significantly increased in high dose males and the thyroid weight was increased in low dose males. Since no collaborative histopathological changes were seen in the tissues, the increases were not considered to be treatment related and were probably due to a decrease in the body weight at termination.

CONCLUSIONS:

Cacodylic acid (99.8%) was administered as a single oral dose by capsule to 4 groups of 5 purebred beagle dogs per sex at dose levels of 0 (Control), 6.5, 16 and 40 mg/kg/day 6 days a week for 52 weeks.

A dose related increase in the diarrhea incidence occurred in both sexes while an increase in the incidence of vomiting occurred in females. Vomiting was increased at the high dose in the male.

Decreases in body weight gain occurred in the high dose males while body weight and body weight gain decreased in high dose females.

High dose level males showed decreases in total protein and albumin at 12, 25, 39 and 51 weeks while high dose females showed decreases in albumin on week 39 and total protein on week 51.

Decreases in HCT, HgB and RBC occurred in the high dose males at weeks 12, 25 (dose related) and 51.

Based on the results of this study, the following NOEL and LOEL are established: NOEL = 16 mg/kg/day; LOEL = 40 mg/kg/day. The LOEL is based on clinical signs, decrease body weight and body weight gain and alterations in clinical chemistry and hematology.

CORE CLASSIFICATION: Minimum

This study satisfies the data requirements (83-1) for a Chronic Toxicity Study and is acceptable for regulatory purposes.