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

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OCT 2 2 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Naptalam - Review of studies submitted in support of reregistration.
EPA DP Barcode D165749, D174281, D174280; EPA Submission No.s S397030, S411304, S411303; MRID #'s 418388-00, 418388-01, 00077053, 410575-01, 00157186, 00031684, 402745-02, 400691-03, 400691-04, 400691-05, 401498-01; HED Project No.s 1-1608, 2-1301, 2-1444; EPA Pesticide Chemical Code 030703, Caswell No. 592 and 780A.

TO: Walter Waldrop/Susanne Cerrelli, PM 71
SRRD (H7508W)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 10/21/92
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y. M. Ioannou
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. Mvan Gemert 10/22/92
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Uniroyal Chemical
Pesticide: Sodium N-1-naphthylphthalamate
Chemical#/Case#: 030703/0183

Action Requested: Review of Naptalam studies.

Recommendations: Toxicology Branch II reviewed the following studies: MRID #'s 418388-01 (additional data for MRID # 410575-01), 00077053, §83-1a, Chronic toxicity/rodent and §83-2a, Carcinogenicity/rat , 410575-01, §83-1b, Chronic toxicity/nonrodent, 00157186, §83-3b, Teratology Study in Rabbits, 00031684, §83-4, Multigeneration Study in Rats, 402745-02, §85-1, General Metabolism (includes 418600-03), 400691-03, §84-2, Category I. Gene Mutations, 400691-04, §84-2, Category II, Structural Chromosome Aberrations, 400691-05, §84-2, Category II, Structural Chromosomal Aberrations, 401498-01, §84-4, Category III, Other Mutagenic Mechanisms.

The following are the conclusions of the reviews:

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MRID # 418388-01, Composition of ALANAP Used in Chronic Toxicity Study Uniroyal Project No. 8969 (for Guideline §83-1b). See discussion under MRID # 410575-01 below.

MRID # 00077053, 104-Week Chronic Toxicity Study in Rats, 6Q8, Na Salt (Alanap Technical), Final Report, Hazleton Laboratories America, Inc., Project No. 798-177, 5/20/81 for Guideline §83-1a, Chronic toxicity/rodent and §83-2a, Carcinogenicity/rat:

Alanap was administered to 50 male and 50 female Sprague-Dawley rats for 2 years at dietary levels of 0, 120, 600, or 3000 ppm (estimated intake of about 5.6, 27, and 140 mg/kg/day). There was no carcinogenic response to dosing. At the highest dose level (3000 ppm), mean body weights and body weight gains in females were decreased 7% and 9%, respectively, compared to controls, but in males, mean weights and weight gains were similar to controls. Survival was not affected by dosing and ranged from 46% to 68% in male groups and from 56% to 62% in female groups at 104 weeks. No clear effects on any clinical parameters, on organ weight data or on macroscopic or microscopic findings were observed in treated rats. The dosing was not considered adequate to test for carcinogenicity. An effect level (LOEL) was not established in either sex; it was greater than the highest dose tested (HDT); the NOEL was equal to or greater than the HDT. Further, the MTD (maximum tolerated dose) was not achieved in this study. The study was classified as Core-Supplementary Data for both chronic toxicity and carcinogenicity and does not satisfy the guideline requirements for §83-1a, Chronic toxicity in the rodent and §83-2a, Carcinogenicity in the rat.

MRID # 410575-01, 12 Month Chronic Oral Toxicity Study in the Dog with ALANAP, Tegeris Laboratories, Inc., Project No. 97002, 3/28/89, for Guideline §83-1b, Chronic toxicity/nonrodent:

ALANAP was fed to dogs for 1 year at dietary levels of 0, 200, 1000, or 5000 ppm (the corresponding doses were estimated as 5.3, 25.8, or 121 mg/kg/day). No observed signs of overt toxicity were related to dosing. Mean weight gains in high-dose dogs of both sexes were about 10% lower than controls. No effects of biological importance on body weight, food consumption, hematologic parameters, or urinary parameters were observed. No effects of dosing on gross or histologic findings were seen. Serum alkaline phosphatase activity and total bilirubin levels were significantly increased in high-dose males and females at 26 and 52 weeks. Significant ($p < 0.05$) increases in liver-to-body-weight ratios were observed in high dose males and mid- and high-dose females (significant at $p < 0.05$ in females). Based on effects on liver weights and increased levels of serum alkaline phosphatase and bilirubin, the LOEL is 5000 ppm in males and 1000

ppm in females. The NOEL is, therefore, 200 ppm. This study was classified as Core-Minimum Data and satisfies the guideline requirements (§83-1b) for a chronic toxicity study in nonrodents.

MRID # 00157186, Technical Alanap (Na Salt), Teratology Study in Rabbits, IRDC, Study No. 399-053, 5/31/85 for Guideline §83-3b, Teratology Study in Rabbits:

This study has been reviewed previously, copy of DER is attached. The following are the conclusions from that review:

The No Observed Effect Level (NOEL) for Maternal Toxicity is 200 mg/kg/day. The Lowest Observed Effect Level (LOEL) for Maternal Toxicity is 650 mg/kg/day based on reduced body weight gain during the dosing period, mortality and clinical observation of reduced amount of stool. The NOEL for Developmental Toxicity is conservatively established at 200 mg/kg/day. The LOEL for Developmental Toxicity is 650 mg/kg/day based on the findings of increased numbers of litters (and fetuses) presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae. However, the low number of dams for evaluation in this dose group may have restricted the adequate interpretation of the data for this group. The study was classified as Core-Minimum Data and satisfies the guideline requirement for a developmental toxicity study in rabbits (§83-3a).

Maternal Toxicity NOEL = 200 mg/kg/day
Maternal Toxicity LOEL = 650 mg/kg/day
Developmental Toxicity NOEL = 200 mg/kg/day
Developmental Toxicity LOEL = 650 mg/kg/day

MRID # 00031684, Multigeneration Evaluation of Alanap Technical in the Sprague-Dawley Rat, Food and Drug Research Laboratories, Inc., Laboratory No. 5847, January 11, 1980 for Guideline §83-4, Multigeneration Study in Rats:

This study was screened under FIFRA 88 and the following conclusions were reached: Based on preliminary assessment of the study, a recommendation is reserved. The study is not acceptable at this time but may be upgraded by submission of the following data: percent active ingredient of the test compound, diet analyses, and age of animals at start of study. The company responded by submitting supplemental data concerning the percent active ingredient of the test compound used in the study, the analysis of the test diet and the age and weight of the animals at the start of the study. Therefore, based on the additional information the study was acceptable for review.

However, according to the Clement reviewers (memo is attached):

> Due to a number of deficiencies in the study, the reviewers opted not to prepared a full DER. The laboratory did not comply with GLP Standards as it was conducted in 1978 prior to establishment of GLP standards by EPA. Several deficiencies (in study design as well as conduct) exist in the study and include, but are not limited to, the following:
>
> > The majority of the control and test animals of F_1 and F_2 generation that were examined microscopically, revealed early chronic lung disease consisting of interstitial inflammation and peribronchial and/or perivascular lymphocytic hyperplasia. Thus, it appears that the performing laboratory did not use healthy animals.
> >
> > No individual data on clinical observations, body weight, and food consumption were provided which prevented assessment of treatment related effects on these parameters. Only weekly mean body weight and food consumption data for premating, gestation, and lactation were provided. In the absence of individual data the health status of each animal cannot be determined.
> >
> > No individual dam records with litter data and data on their reproductive parameters were provided. Instead, only summary data were reported which made it impossible to assess the effects on reproduction and to verify the statistical significance of the reported findings.
> >
> > There were several deviations from the protocol during the conduct of the study (for example checking for evidence of mating, i.e. vaginal plug or presence of sperm, was not properly conducted).
> >
> > Histopathology examination on F_0 parental tissues was not conducted.
> >
> > The provided information is insufficient to make a judgement regarding the potential reproductive toxicity of Naptalam. Therefore, we have tentatively classified the study as

invalid and further recommend that the missing individual animal data be submitted to EPA so that a comprehensive analysis of data can be made. Thus, based on our preliminary review of the study, we conclude that no independent evaluation can be made.

MRID # 402745-02, Analysis of Urine Samples From [^{14}C] Naptalam (ALANAP) Rat Metabolism Study, Uniroyal Chemical Co., Inc. and Biotek, Inc., Uniroyal Project No. 8660 and Biotek Study No, 8603B, 7/7/87 (7/10/87) for Guideline §85-1, General Metabolism, includes MRID # 418600-03, Analysis of Percent Active Ingredient of the Test Compound:

The absorption, distribution, metabolism, and excretion of naptalam were studied in groups of male and female CD rats administered a single oral dose of 250 or 1000 mg/kg [^{14}C] naptalam by gavage.

[^{14}C]Naptalam was rapidly absorbed, distributed, and excreted in rats at both dose levels. The 7-day recoveries were at least 84.85% of the administered dose for all dosing groups, with higher recoveries in the males. The elimination of radioactivity in the urine (39.39-45.30%) was almost comparable for all male and female dose groups. The radioactivity in the feces was 59.13-67.73% in male groups and 41.56-43.10% in the female groups. The elimination data suggest that absorption of naptalam is rapid, bioaccumulation is low, and excretion occurs in the feces and urine. The authors concluded that most radioactivity in the feces was due to unabsorbed test material because most of the fecal elimination occurred within 24 hours. This explanation could not be confirmed because intravenous dosing was not conducted to provide further information on the fecal and urinary elimination pattern. The urine contained one major radioactive band which was identified as the unmetabolized parent compound. No metabolites were identified in the urine. Since the metabolism of naptalam was not evaluated in the feces, the complete metabolite pattern of naptalam in rats cannot be determined. The study also indicated that naptalam and/or its metabolites do not bioaccumulate to an appreciable extent following oral exposure since all the tissues contained negligible levels of radioactivity at 7 days postexposure.

Based on the study results, absorption, distribution, and elimination of naptalam do not appear to be sex or dose related. No conclusion can be made regarding sex- or dose-related differences in the metabolism of naptalam since radioactivity in the feces was not analyzed. Furthermore, no metabolic pathway could be determined for naptalam. The study also showed that administration of 250 and 1000 mg/kg naptalam did not induce any

apparent treatment-related clinical effects.

The study is classified as **Core-Supplementary Data** and **does not satisfy** the guideline requirement (§85-1) for a General Metabolism study in Rats. This study may be upgraded if the following additional data are provided:

1) identification of metabolites in the feces to evaluate sex-related differences in metabolism and determine metabolic pathway of naptalam;

2) intravenous dosing study to evaluate fecal elimination (i.e., unabsorbed test material and/or biliary excretion);

3) repeated dosing study to evaluate toxicokinetic differences with different dosing regimen.

MRID # 400691-03, CHO/HGPRT In Vitro Mammalian Cell Mutation Assay on Sodium Alanap, American Biogenics Corp., Project No. 8600 15-30, 1/5/87 (1/28/87) for Guideline §84-2, Category I. Gene Mutations:

Sodium alanap at nonactivated doses ranging from 100-1500 µg/mL and S9-activated doses of 14.9-996 µg/mL did not induce a mutagenic response in CHO cells at the HGPRT locus. The study was, however, seriously compromised because of the marked differences in the cytotoxicity data obtained from the preliminary and mutational assays. For example, relative percent survival (RPC) was 50 and 56% after exposure to 2860 µg/mL -S9 and 1000 µg/mL +S9, respectively in the preliminary test. By contrast, ~8% of the cells were recovered at 1500 µg/mL -S9 and <1% survived treatment with ≈1000 µg/mL -S9 in the mutation assay. Since the test material was reported to be stable, the reviewers assumed that the inability to reproduce reasonably comparable cytotoxicity results probably resulted from substandard culture conditions rather than test material instability. The poor cloning efficiency of the background control cultures (~50% -S9, 44.7% +S9--preliminary cytotoxicity test; 55.5% -S9, 43.4% +S9--mutation assay) supports this assessment. In addition, the purity of the test material was not provided. Based on these considerations, it was concluded that the study is classified as **Unacceptable and does not** fulfill the data requirements for Guideline §84-2 for genetic effects, Category I, Gene Mutations and should be repeated.

MRID # 400691-04, In Vitro Chromosomal Aberration Assay on Sodium Alanap, American Biogenics Corp., Project No. 860015-20, 12/8/86 (1/28/87) for Guideline §84-2, Category II, Structural Chromosome Aberrations:

Sodium alanap at 771, 1540, and 2570 µg/mL +S9 induced a dose-related clastogenic response in Chinese hamster ovary cells following a 2-hour treatment and a 17-hour recovery time. The dramatic increase in the aberration frequency at 2570 µg/mL +S9 (i.e., 83% of the cells with simple and complex aberrations) was accompanied by cytotoxicity. In the absence of S9 activation, 15% of the cells exposed to the highest scorable level (1490 µg/mL for 17 hours) had chromosome aberrations (simple and complex). The overall findings clearly indicate that the use of a prolonged cell harvest was necessary to demonstrate the clastogenesis of sodium alanap. Although a direct comparative evaluation of the nonactivated and S9-activated results is not possible because of the different treatment periods, the data provided convincing evidence that sodium alanap is clastogenic in cultured CHO cells. The study is, nevertheless, incomplete because information on the purity of the lot of the test material used in this assay was missing.

This study is classified as **Unacceptable and does not satisfy** the guideline requirements for a mutagenicity study for genetic effects, Category II, Structural Chromosome Aberrations (§84-2) but can be upgraded if the test material purity information is submitted. Sodium alanap is, however, classified as positive in this *in vitro* mammalian cell cytogenetic assay.

MRID # 400691-05, Micronucleus Assay with Sodium Alanap, American Biogenics Corp., Project No. 860015-10, 12/6/86 (1/28/87) for Guideline §84-2, Category II, Structural Chromosomal Aberrations:

No definitive conclusions can be reached from the mouse micronucleus assay conducted with males and females administered a single oral gavage dose of 1500 mg/kg sodium alanap. The data suggested a time-related increase in micronucleated polychromatic erythrocytes (MPEs) with a maximum response occurring 2 days postexposure in the females and 3 days postexposure in the males. Without historical background frequencies, it is impossible to determine whether the increases in MPEs fell within the normal range for the reporting laboratory or are indicative of a genotoxic response. It was noteworthy, however, that the increase in MPEs over time was consistent with the equivocal evidence of sodium alanap-induced cell-cycle delay and clastogenesis in cultured Chinese hamster ovary (CHO) cells (above). The interpretation of the data was further confounded by the overall lack of agreement between the LD_{50}, the preliminary toxicity test,

and the micronucleus assay survival data. The results, therefore, did not provide full assurance that doses were accurately prepared or that animals were treated with an appropriate concentration of the test substance. Additionally, there was no information on the purity of the test material. Based on the above considerations, the study was classified as Unacceptable and does not satisfy the guideline requirements (§84-2) for a mutagenicity study, Category II, Structural Chromosomal Aberrations. It was recommended by the reviewers that the repeated assay be conducted with a high level that clearly demonstrates the maximum tolerated dose for both sexes. In light of the suggestive evidence of a time-related increase in MPEs, it is further recommended that three doses be assayed for the determination of a dose-dependent response. The study report should also include information on test material on test material purity and all clinical signs including death.

MRID # 401498-01, Unscheduled DNA Synthesis in Rat Primary Hepatocytes Test Article Sodium ALANAP, Microbiological Associates, Inc., Laboratory Study No. T5270.380, 3/4/87 (3/31/87) for Guideline §84-4, Category III, Other Mutagenic Mechanisms:

Concentrations ranging from 3 to 300 µg/mL sodium alanap did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. Doses ≥100 µg/mL were insoluble and levels ≥1000 µg/mL were severely cytotoxic. It was concluded, therefore, that sodium alanap was tested over an appropriate range of concentrations and failed to induce a genotoxic response. Although the assay was performed in a technically acceptable manner, the study is incomplete because information on the purity of the lot of sodium alanap used in the study was not provided. The study was classified **Unacceptable and does not** satisfy the guideline requirements (§84-4) for genetics effects, Category III, Other Mutagenic Mechanisms but can be upgraded if the missing test material purity information is provided.

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FINAL

DATA EVALUATION REPORT

NAPTALAM

Study Title:
104-Week Chronic Toxicity Study in Rats
6Q8, Na Salt (Alanap Technical)

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

September 30, 1992

Principal Reviewer: William L. McLellan Date Sept 30, 1992
William L. McLellan, Ph.D.

Independent Reviewer: John Liccione Date 9/30/92
John Liccione, Ph.D.

QA/QC Manager: Sharon Segal Date 9/30/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-71
Project Officer: James E. Scott

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Guideline 83-5: Combined Chronic/Oncogenicity Feeding Study in Rats

EPA Reviewer: Stephen C. Dapson, Ph.D. Signature: Stephen C. Dapson
Review Section I, Toxicology Branch II,
Health Effects Division Date: 10/1/92

EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Y. M. Ioannou
Section Head, Toxicology Branch II,
Health Effects Division Date: 10/21/92

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Study in Rats

TEST MATERIAL: Alanap Technical

EPA Pesticide Chemical Code: 030703

Tox Chem. Number: 780A and 592

MRID Number: 000770a53, ~~41880001~~ 41848501

SYNONYMS: 6Q8 (Na Salt)

PROJECT NUMBER: 798-177

SPONSOR: Uniroyal Chemical
Bethany, CT

TESTING FACILITY: Hazleton Laboratories America, Inc.
Vienna, VA

TITLE OF REPORT: 104-Week Chronic Toxicity Study in Rats
6Q8, Na Salt (Alanap Technical)

AUTHORS: Serota, D. G.; Alsaker, R.D.; Dawkins, K.K.; Kundins, W.

REPORT ISSUED: May 20, 1981

CONCLUSIONS: Alanap was administered to 50 male and 50 female Sprague-Dawley rats for 2 years at dietary levels of 0, 120, 600, or 3000 ppm (estimated intake of about 5.6, 27, and 140 mg/kg/day).

There was no oncogenic response to dosing. At the highest dose level (3000 ppm), mean body weights and body weight gains in females were decreased 7% and 9%, respectively, compared to controls, but in males, mean weights and weight gains were similar to controls. Survival was not affected by dosing and ranged from 46% to 68% in male groups and from 56% to 62% in female groups at 104 weeks. No clear effects on any clinical parameters, on organ weight data, or on macroscopic or microscopic findings were observed in treated rats. The dosing was not considered adequate to test for oncogenicity. An effect level (LOEL) was not established in either sex; it was [redacted] greater than the highest dose tested (HDT); therefore, the NOEL cannot be determined. Further, the MTD (maximum tolerated dose) was not met in this study.

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CORE CLASSIFICATION: The study is considered Core Supplementary for both chronic toxicity and carcinogenicity. Several of the study deficiencies are presented in Section C--Reviewers' Discussion and Interpretation of Results.

A. MATERIALS AND METHODS

1. Test Article Description

Name: 6Q8 Na Salt (Alanap Technical)

Lot number: 3199300 E604

Purity: 92.6% Sodium Alanap by analysis

Physical property: Pale pink powder

Stability: Not reported

2. Diet Preparation

Premixes were prepared by weighing an appropriate amount of diet and test material and mixing in a Waring blender. Appropriate amounts of basal diet were then added to the premixes and mixed in a Patterson-Kelly blender (1 minute/kg diet) in order to obtain appropriate dose levels. Diets were prepared weekly.

Results: A supplemental report (MRID No. 418388-01), dated April 5, 1991, presented analytical data for the test compound. The material was 90.48% pure. The major impurity was water; sodium phthalate and α-napthylanine each were less than 1.10% of the sample. A supplemental report (MRID No. 418600-01) dated April 3, 1991, with a signed and dated QLP statement provided information on analysis of test compound in diet. The methodology had a recovery of 101 to 102% for spiked samples. Stability analysis indicated 82 to 85% recovery after 7 or 14 days storage of a diet fortified at 50 ppm. Homogeneity assays at week 82 gave ranges of 76-115%, 87-99%, and 70-82% of target for samples at three levels of the mixer for diets with target levels of 120, 600, or 3000 ppm. Mean (±SD) for analyses of diets at 13 intervals at nominal levels of 120, 600, or 3000 ppm, respectively, were 90.6±15.1%, 97.2±13.0%, and 98.8±12.4% of target.

3. Animals

Species: Rat

Strain: Sprague-Dawley

Age: Weanling

Weight at initiation: 94-190 g (group means) for males and 100-190 g (group means) for females

Source: Charles River Breeding Laboratories, Inc., Wilmington, MA

Animals were acclimated to laboratory conditions for 16 days and were assigned to the following groups using a computerized randomization program:

Test Group	Dietary Level (ppm)	Main Study (24-months)	
		Males	Females
1 Control	0	50	50
2 Low-dose (LDT)	120	50	50
3 Mid-dose (MDT)	600	50	50
4 High-dose (HDT)	3000	50	50

Animals were examined for health status prior to study initiation. They were caged individually during the study. Environmental conditions were not reported.

Rationale for dose selection: Not provided.

4. Statistics

Body weight gains for individual animals were compiled for males at weeks 2, 4, 8, 17, and 51 and for females at weeks 2, 5, 11, 31, and 51. Weight gain, food consumption, and appropriate clinical laboratory data were analyzed by Bartlett's test and ANOVA (one way) for homogeneity of data. For homogeneous data, pairwise comparisons to controls were performed for means, and for nonhomogeneous data (with Bartlett's test), Scheffe's multiple comparison procedure was used. Organ weight data were similarly analyzed, and nonhomogeneous data were transformed by $\log_{10}$ or $\log_e$. Survival data were analyzed by Sach's life-table technique.

5. Compliance

Quality assurance statement, confidentiality statement, compliance with GLP's statement, and flagging statement were not provided.

B. METHODS AND RESULTS

1. General Observations

All animals were observed twice daily for mortality and signs of moribundity. Animals received detailed physical examinations (including palpations) weekly for 13 weeks, biweekly for weeks 14-27 and weeks 96-103, and every 4 weeks for weeks 28-95. Gross observations and clinical signs of toxicity were recorded at the above intervals.

Results: Table 1 indicates cumulative deaths/moribund sacrifices and percent survival at weeks 51, 79, and at study termination. No effects of dosing on survival were observed. Survival at 105-106

weeks ranged from 46% to 64% in male groups and 54% to 62% in female groups.

No distinct treatment-related signs of toxicity were reported in animals that died during the study or in those that survived to terminal sacrifice. Antimortem signs (thinness, hunched appearance, labored respiration, anorexia, urine staining of fur, and red crusty eyes) occurred at comparable rates in dosed and control groups. Rats that survived to week 104 had similar signs, additional occurrence of sores and swelling in the extremities, tail, and body, and ocular changes. Suspected neoplasms (nodules, masses and wart-like lesions) were more frequent in females than in males, but no dose-related trends or increases were apparent. The following incidences of tissue masses were seen:

Test Group	Dose in Diet (ppm)	Incidence of Tissue Masses	
		Males	Females
1 Control	0	2	27
2 Low-dose (LDT)	120	9	32
3 Mid-dose (MDT)	600	6	31
4 High-dose (HDT)	3000	5	22

2. Body Weights/Food and Water Consumption/Test Material Intake

Body Weights

Body weights were recorded weekly until week 13, then they were recorded biweekly until week 27, and once every 4 weeks thereafter. Weight change data were determined for weeks 0-51 and 51-104.

Results: Table 2 presents mean body weights and body weight gains at representative intervals. At week 51, mean body weights in high-dose males were 2% lower than in controls and weight gains were decreased 3%. In dosed females, mean weights and weight gains were slightly higher than controls for the low-dose and mid-dose groups; mean weights at the high-dose were 7% lower than in the controls ($p<0.05$); and mean weight gains were 9% lower than in controls ($p<0.05$).

Food and Water Consumption

Food (Purina® Rodent Laboratory Chow) and water were available *ad libitum*. Food consumption was determine at the same time intervals as body weights.

Results: Normal variations in weekly food consumption were observed, but no effect of dosing on food consumption was observed.

Water consumption was not monitored. Food efficiency data were not provided.

Test Material Intake

Data were not provided. However, based on analytical levels in the diets, food consumption values, and mean body weights at week 52, the compound intake in males was approximately 5.6, 27, and 140 mg/kg/day (calculated by the reviewers) at nominal dietary levels of 120, 600, or 3000 ppm, respectively.

3. Ophthalmoscopic Examination

No data were presented.

4. Clinical Pathology

Blood was collected from five rats/sex/group at 13, 26, 52, 78, and 104 weeks for clinical laboratory tests. For hematology, samples were collected from the tail; for clinical chemistry, samples were collected from the tail (week 13), or by orbital sinus puncture (weeks 26, 52, and 78), or from the abdominal aorta (week 104). The checked (X) parameters were examined.

(a) Hematology

X Hematocrit*	X Leukocyte differential count
X Hemoglobin (HGB)*	Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	Mean corpuscular volume (MCV)
X Platelet count*	Coagulation: thromboplastin time (PT)
Reticulocyte count (RETIC)	
Red cell morphology	

*Recommended by Subdivision F (November 1984) Guidelines

Results: No clearly compound-related effects on hematology parameters were observed. Table 3 summarizes data for males on red cell parameters. The mean values for hematocrit (HCT), hemoglobin (HGB), and erythrocyte (RBC) counts were slightly depressed for high-dose males at weeks 58 and 78 and for mid-dose males at week 78, when compared with controls. There were no corresponding effects in females and no marked effects in males at 104 weeks. At weeks 78 and 104, morphological blood cell changes were noted but the changes were randomly scattered among groups and not considered compound related.

(b) Blood (Clinical) Chemistry: The checked (X) parameters were examined on 5 rats/sex/group only at week 104. The double-checked (XX) parameters were examined on 5 rats/sex/group at weeks 13, 26, 52, 78, and 104.

Electrolytes

X Calcium*
Chloride*
Magnesium*
Phosphorus*
X Potassium*
Sodium*

Enzymes

XX Alkaline phosphatase (ALP)
Cholinesterase
Creatine phosphokinase
X Lactic acid dehydrogenase
XX Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other

XX Albumin*
XX Albumin/globulin ratio
XX Blood creatinine*
XX Blood urea nitrogen*
X Cholesterol [total]*
XX Globulin
XX Glucose (fasting)*
XX Total bilirubin*
Direct bilirubin
XX Total protein*
Triglycerides

*Recommended by Subdivision F (November 1984) Guidelines

Results: No biologically important effects of dosing were observed for the clinical chemistry parameters examined. The study authors suggested that the decreased mean levels of lactic acid dehydrogenase seen in mid- and high-dose males were probably related to dosing.

(c) Urinalysis

Appearance*	X Sediment (microscopic)	X Bilirubin*
Volume*	X Protein*	Blood
X Specific gravity*	X Glucose*	Nitrate
X pH*	X Ketones	Urobilinogen

*Recommended by Subdivision F (November 1984) Guidelines

Results: No effects of dosing on any urinary parameters were observed.

5. Sacrifice and Pathology

The checked (X) tissues were preserved for histological examination.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*[a]	X Brain (3 levels)[b]
X Salivary glands*	X Heart*[b]	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (two levels)[a]
X Stomach*	X Lymph nodes*	X Pituitary*[c]
X Duodenum*	X Spleen[b]	X Eyes (with Harderian glands)
X Jejunum*	X Thymus	
X Ileum*		Glandular
Cecum*	Urogenital	
X Colon*		X Adrenals*[c]
Rectum	X Kidneys*[b]	Lacrimal gland
X Liver*[b]	X Urinary bladder*	X Mammary gland
Gallbladder*	X Testes*[b]	X Thyroids*[c]
X Pancreas*	X Epididymides[b]	X Parathyroids*
	X Prostate	X Harderian glands
Respiratory	X Seminal vesicle[a]	
	X Ovaries	
X Trachea*	X Uterus	
X Lung*		

Other

X Bone marrow (sternum)*
X Skeletal muscle*
X Skin
X All gross lesions and masses
Nasal septum

*Recommended by Subdivision F (November 1984) Guidelines

[a]Organ was preserved but did not undergo histopathology
[b]Organ was weighed before fixation
[c]Organ was weighed after fixation

(a) Organ Weights

Table 4 summarizes data on mean pituitary weights at study termination. The absolute and relative (to body) weights were increased in mid- and high-dose males and in high-dose females when compared to controls; the increases were not significant. No compound-related effects on weights of other organs were noted.

(b) Macroscopic Pathology

No treatment-related findings were observed. Frequently occurring findings which were seen at similar incidences in all groups included enlarged and dark red pituitary, red areas in the lungs, enlarged liver, spleen, kidneys, and adrenals, ulcerated areas in the stomach, and thickened mammary glands (females). The incidences

were not markedly higher than normally experienced in chronic studies in rats (data not presented).

(c) Microscopic Pathology

Summary tabulations of nonneoplastic findings were not provided. Scanning of the individual animal histopathology tabulations (by the reviewer) for animals that died and were sacrificed moribund or those sacrificed at termination did not reveal any dose-related increase in incidence or severity of findings.

Table 5 summarizes the frequency of neoplasms. No compound-related increase in neoplastic findings was observed. Incidences of neoplasms were within the range normally expected for rats, and any increased incidences (e.g., astrocytoma of brain in 3/50 high-dose males compared to 1/50 control males) were related to normal biologic variation. No oncogenic response was seen.

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The study had a number of deficiencies:

- Clinical chemistry parameters were determined for only five animals/sex/group and only on six of the required parameters at all intervals of blood sampling. The only electrolytes measured were calcium and potassium at 104 weeks. Cholesterol and SGOT were also measured only at 104 weeks.

- Sufficient hematology parameters were analyzed to meet minimum guideline requirements, but only five animals/sex/group were used.

- Compound intake was not calculated.

- Individual animal body weights were not provided.

- A protocol was not included.

- No ophthalmologic examination data were provided.

- The summary tabulations of histologic findings did not indicate the number of tissues examined; the reviewer provided the number derived from individual animal tabulations.

- Nonneoplastic findings were not summarized.

- The tabulations of histologic findings graded the severity of only a few lesions; the entry P (present) was entered for most.

- No individual animal gross findings were provided; therefore, correlations could not be made with in-life masses or with histologic diagnoses.

- Several pages of tabulated data were not fully legible.

The rats should have been able to tolerate a higher dose than they were administered. A LOEL was not achieved in either sex; body weight gains were decreased only 3% for males and 9% for females compared to controls; and no toxicologically important effects on any parameters were observed.

TABLE 1. Cumulative Mortality (percent survival) in Rats Fed Alanap for 104 Weeks[a].

Dietary Level (ppm)	Morralities (percent survival) at: Week 51	Week 79	Termination
		Males	
0	4 (92)	9 (82)	27 (46)
120	0 (100)	5 (90)	27 (46)
600	1 (98)	8 (84)	26 (48)
3000	2 (96)	8 (84)	18 (68)
		Females	
0	1 (98)	5 (90)	20 (60)
120	0 (100)	5 (90)	19 (62)
600	1 (98)	6 (88)	22 (56)
3000	1 (98)	7 (86)	21 (58)

[a]Data extracted from Study No. 798-177, Table 1, pp. 24-27.

TABLE 2. Mean Body Weight Data in Rats Fed Alanap for 104 Weeks[a]

Dietary Level (ppm)	Mean Body Weight (g±SD) at Week						Mean Body Weight Gain and (% Change) at Weeks[b]		
	0	13	25	51	79	104	0–13	0–51	0–104
Males									
0	148±14.0	518±39.4	584±49.6	665±57.4	674±58.9	596±134.7	370	517	448
120	148±17.6	514±43.6	582±55.3	653±64.4	640±73.1	577±107.8	366 (-1.1)	505 (-2.4)	429 (-4.3)
600	150±18.2	515±52.5	587±56.8	658±63.3	653±72.0	609±95.9	365 (-1.4)	508 (-2.8)	459 (+2.4)
3000	151±14.6	506±37.2	577±43.3	653±58.1	656±83.7	584±95.9	355 (-4.1)	502 (-3.0)	433 (-3.4)
Females									
0	131±11.2	273±24.7	302±30.9	358±49.6	411±59.5	421±78.3	142	227	290
120	128±11.6	272±22.8	305±26.8	363±49.6	408±57.5	425±72.1	144 (+1.4)	235 (+3.5)	297 (+2.4)
600	128±10.8	274±27.3	310±35.8	367±58.3	405±69.4	424±79.8	146 (+2.8)	239 (+5.3)	296 (+2.1)
3000	127±11.1	259±21.9	288±26.1	334*±30.0	392±48.0	394±82.0	132 (-7.0)	207 (-8.9)	267 (-8.0)

[a]Data extracted from Study No. 798-177, Table 1, pp. 24-27

[b]These data are the differences between mean values. Since individual animal body weights were not provided, means of gains based on individual animal weight gains could not be calculated.

*Significantly different from control values ($p<0.05$)

TABLE 3. Mean Hematology Data for Male Rats Fed Alanap for 104 Weeks[a,b]

Parameter and Dietary Level (ppm)	Mean Hematology Data at Week:				
	13	26	52	78	104
Hematocrit (%)					
0	47.4±1.2	46.6±1.1	44.2±5.4	44.3±1.5	36.5±12.
120	45.6±2.3	47.0±1.2	45.2±0.8	44.8±1.2	37.6±4.9
600	46.1±2.1	45.8±2.6	45.6±1.7	38.1±5.0	43.2±3.1
3000	45.7±4.0	46.0±2.4	40.9±12.1	37.2±12.1	40.0±1.6
Hemoglobin (g/dL)					
0	16.2±0.3	15.0±0.7	14.5±1.5	14.4±0.8	12.0±4.7
120	15.8±0.7	15.1±0.4	14.3±0.5	15.0±0.3	11.8±2.2
600	15.8±0.7	14.2±0.9	14.6±0.6	13.0±2.0	14.4±1.7
3000	15.8±1.4	14.5±1.0	12.5±4.4	12.0±4.6	12.8±1.1
Erythrocyte Count					
0	8.1±0.4	8.4±0.7	7.3±1.1	7.5±0.3	6.3±2.2
120	8.1±0.6	8.2±0.2	7.8±0.9	7.9±0.4	6.1±1.0
600	8.1±0.4	7.6±0.5	7.4±0.2	7.1±0.8	7.1±0.7
3000	7.8±0.4	7.6±0.3	6.5±2.3	6.4±2.2	5.9±1.1

[a]Data extracted from Study No. 798-177, Table 2, pp. 32-34

[b]Based on five animals/sex/group

TABLE 4. Mean Pituitary Weight and Pituitary/Body Weight Ratio in Rats Fed Alanap for 104 Weeks[a]

Dietary Level (ppm)	Mean (g ± S.D.)		
	Body weight (g)	Pituitary Weight (g)	Ratio (%)
		Males	
0	591±113	0.030±0.020	0.0052±0.0030
120	570±105	0.037±0.084	0.0081±0.022
600	598± 92	0.045±0.089	0.0111±0.032
3000	573± 92	0.049±0.066	0.0095±0.014
		Females	
0	392±71	0.12±0.11	0.035±0.038
120	413±96	0.12±0.15	0.032±0.047
600	397±76	0.10±0.11	0.028±0.032
3000	364±89	0.16±0.19	0.059±0.084

[a]Data extracted from Study No. 798-177, Table 4, pp. 44-52

TABLE 5. Frequency of Neoplasms in Rats Fed Analap Technical for 104 Weeks[a].

Tissue/ Neoplasm	Dietary Level (ppm) Males 0	120	600	3000	Females 0	120	600	3000
Brain								
Astrocytoma	1	0	1	3	1	2	1	2
Pituitary								
Adenomas	18	23	20	24	40	38	41	38
Carcinoma	7	2	2	1	1	4	3	3
Thyroid								
C-cell adenoma	2	4	0	2	2	1	2	3
Adrenals								
Cortical cell								
Carcinomas	2	1	0	0	3	1	1	0
Adenomas	1	1	0	0	9	1	0	3
Pheochromocytoma	8	3	6	5	2	3	0	0
Liver								
Neoplastic nodule	1	2	1	3	6	0	1	5
Hepatocellular carcinomas	2	3	4	4	1	1	3	2
Testis								
Interstitial cell tumor	2	3	3	2	-	-	-	-
Mesothelioma	0	0	1	2	-	-	-	-
Mammary gland								
Adenoma	-	-	-	-	0	0	1	2
Fibroadenoma	-	-	-	-	16	14	18	13
Adenocarcinoma	-	-	-	-	9	6	7	9
Pancreas								
Islet cell								
Adenoma	3	2	5	4	1	1	1	0
Carcinoma	0	3	2	0	2	1	0	0
Reticuloendothelial system								
Malignant lymphomas	0	0	0	1	0	1	2	1
Fibrous histocytoma, malignant	1	0	1	1	4	2	1	0
Uterus								
Endometrial stromal polyp	-	-	-	-	2	3	1	2

[a]Data extracted from Study No. 798-177, Text Table pp.19-22 and Tables 5A and 5B

[redacted] Doc c930172

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FINAL

DATA EVALUATION REPORT

NAPTALAM

Study Title:

12-Month Chronic Oral Toxicity Study in the Dog
with ALANAP

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

September 30, 1992

Principal Reviewer: William L. McLellan Date Sept 30, 1992
William McLellan, Ph.D.

Independent Reviewer : John Liccione Date 9/30/92
John Liccione, Ph.D.

QA/QC Manager: Sharon A. Segal Date 9/30/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-70
Project Officer: James E. Scott

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Guideline Series 83-1: Chronic Toxicity in Dogs

EPA Reviewer: Stephen C. Dapson, Ph.D.
Review Section I, Toxicology Branch II,
Health Effects Division
Signature: Stephen C. Dapson
Date: 10/1/92

EPA Section Head: Yiannakis M. Ioannou, Ph.D.
Review Section I, Toxicology Branch II,
Health Effects Division
Signature: Y M. Ioannou
Date: 10/21/92

DATA EVALUATION REPORT

STUDY TYPE: 12-Month Dietary Toxicity Study in Dogs

TEST MATERIAL: ALANAP Technical

EPA Pesticide Chemical Code: 030702

Tox Chem. Number: 592 and 780A

MRID Number: 41056[7]5-01 [40]

SYNONYMS: Naptalam; Sodium N-1-naphthylthalamate; 2-[(1-naphthalenylamino) carbonyl]benzoic acid, Na salt

STUDY NUMBER: 97002

SPONSOR: Uniroyal Chemical Company Inc., Bethany, CT

TESTING FACILITY: Tegeris Laboratories Inc., Laurel, MD

TITLE OF REPORT: 12-Month Oral Toxicity Study in the Dog with ALANAP

AUTHOR: A.S. Tegeris

REPORT ISSUED: March 28, 1989

CONCLUSIONS: ALANAP was fed to dogs for 1 year at dietary levels of 0, 200, 1000, or 5000 ppm (the corresponding doses were estimated as 5.3, 25.8, or 121 mg/kg/day). No observed signs of toxicity were related to dosing. Mean [40] weight gains in high-dose dogs of both sexes were about 10% lower than controls. No effects of biological importance on body weight, food consumption, hematologic parameters, or urinary parameters were observed. No effects of dosing on gross or histologic findings were seen. Serum alkaline phosphatase activity and total bilirubin levels were significantly increased in high-dose males and females at 26 and 52 weeks. Significant ($p < 0.05$) increases in liver-to-body-weight ratios were observed in high-dose males and

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mid- and high-dose females (significant at $p < 0.05$ in females). Based on the effects on liver weights and increased levels of serum alkaline phosphatase and bilirubin, the LOEL is 5000 ppm in males and 1000 ppm in females. The NOEL is 200 ppm.

CORE CLASSIFICATION: Core Minimum. The study satisfies the requirements (83-1, Subdivision F Guidelines, 1984) for a chronic study in nonrodents.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: ALANAP (N-1-Naphthylphthalamic acid)

Lot Number: DJS-1-65A

Purity: 96.48% (see CBI Appendix for impurities)

Physical Property: Purple powder with an aromatic amine odor

Stability: Not reported

2. Diet Preparation

Diets containing the test material were prepared weekly. Premixes of feed and test compound were prepared by homogenating a small quantity of both test material and basal diet in a Waring high-speed blender and then mixing with 3 kg of feed in an open bowl Hobart mixer for 10 minutes. The premix was then added to an appropriate amount of food (about 20 kg) for each group and mixed in a Patterson-Kelley V-shaped mixer for 15 minutes. Prior to study initiation, the homogeneity of test material in feed was determined by analyzing samples from the top, middle, and bottom of the mixer. Stability of test compound in diets was analyzed after storage of treated diets at room temperature for 7 or 14 days. Samples were analyzed for content weekly for the first 4 weeks and monthly thereafter.

Results: Homogeneity was acceptable; coefficients of variation for samples from the mixer were 5.1%, 3.6%, and 2.1% at nominal levels of 0, 200, 1000, and 5000 ppm. The test compound was essentially stable in the diets stored at room temperature for 7 or 14 days. The mean concentrations in the diets at 15 intervals of analysis were 97.8% ± 7.9%, 97.1% ± 8.6%, and 101.4% ± 3.6% percent of nominal at target doses of 200, 1000, and 5000 ppm.

3. Animals

Species: Dog

Strain: Beagle

Age and weight at initiation: Approximately 6 months old; males -- 5.4-9.3 kg;females -- 6.0-8.0 kg

Source: Laboratory Research Enterprises, Kalamazoo, MI

Animals were acclimated to laboratory conditions for 3 weeks. They were selected based on criteria including normal ophthalmoscopic examination, clinical chemistry, hematology, and urinary parameters and were assigned to the following groups after randomization according to body weights with care taken to avoid litter mates of the same sex in the same dose group:

Group	Dietary Level (ppm)	Number of animals	
		Males	Females
1. Control	0	4	4
2. Low dose (LDT)	200	4	4
3. Mid dose (MDT)	1000	4	4
4. High dose (HDT)	5000	4	4

Dogs were vaccinated against Bordetella, parainfluenza, parvovirus, canine distemper hepatitis, and leptospirosis. They received veterinary examinations on arrival and were examined daily for health during quarantine. Fecal examinations were conducted for intestinal parasites. The dogs were housed in stainless steel cages in an environmentally controlled room with a mean temperature of 72°F, relative humidity of 30-70%, and a 12-hour light/dark cycle.

4. Statistics

Food consumption, body weight, clinical chemistry, hematology data, and organ weights (absolute and relative to body and brain weights) were analyzed by ANOVA using the F-test for comparison of variance. If there were significant differences among means, Dunnett's t test was used (95% confidence limits) for pairwise comparison of means of treated groups to control.

5. Compliance

A confidentiality claims statement (no confidentiality claimed) was signed and dated March 28, 1989. A quality assurance statement was signed and dated December 15, 1988. Acompliance with GLP's statement was signed. A Flagging statement for possible adverse effects was

signed and dated March 29, 1989, no adverse effects noted.

B. METHODS AND RESULTS

1. General Observations

(a) Mortality/moribundity/survival

All animals were observed twice daily for clinical signs (appetite, appearance, behavior, excretory functions, and discharges) and mortality. Detailed examinations of each dog were conducted weekly for 13 weeks and monthly thereafter.

Results: Some males and some females in all groups had alopecia and skin cuts, interdigital cysts of the foot, and redness of the ears. Vomiting, loose stools, or diarrhea were observed, but the incidences were infrequent and not considered related to dosing. No adverse clinical signs were observed and no mortalities occurred.

(b) Body weights/food consumption/test material intake

Body weights

Weight data were recorded weekly for 13 weeks and every 4 weeks thereafter.

Results: Table 1 summarizes body weight data. No statistically significant effects were observed on mean body weights. The mean body weights in high-dose males were consistently slightly lower than those of controls; decreases from control values were 5%, 7.4%, and 11.5% at weeks 6, 13, and 25. Mean body weights in mid- and high-dose females were consistently lower than control weights, but the differences were not significant ($p > 0.05$). Mean weights of high-dose females were decreased 5.0%, 7.4%, and 11.5% compared to controls at weeks 6, 13, and 25, respectively.

Weight gain data were not provided in the report. The reviewers calculated weight gain at 13 and 53 weeks from the individual animal data. The data were normalized for the pretest body weight by calculating the gain as percent of pretest values for weeks 13 and 53. The means of the percentages for individual animals for each group/sex were calculated and are presented in Table 1. The mean percent gains in high-dose males were 7% lower than in controls at 13 and 53 weeks and in high-dose females the gains were 11% and 10% lower than controls at 13 and 53 weeks, respectively.

Food consumption and Compound intake

Dogs received 400 g of feed (Purina® Certified Canine Meal No. 5007) for 1 hour each day. Water was provided ad libitum. Food consumption data were reported weekly as individual average daily consumption values.

Results: No compound-related effects on food consumption were observed. Slight increases or decreases were observed in various groups, but they were not consistent with time or dose.

Based on mean body weight and food consumption data at 13 weeks and the nominal concentrations of test compound in the diets, the reviewers estimated the compound intake as 5.3, 28.5, and 121 or 158 mg/kg/day (males or females) at dietary levels of 200, 1000, and 5000 ppm ALANAP, respectively.

(c) Ophthalmoscopic examination

All dogs were examined prior to initiation and prior to the terminal sacrifice. Eyes were examined by indirect ophthalmoscopy.

TABLE 1. Mean Body Weight at Selected Intervals for Dogs Fed ALANAP for 12 Months*

Dietary Level (ppm)	Mean Body Weight (kg ± S.D.) at:					Weight Gain & (Mean Percent of Pretest) at:	
	0 Weeks	6 Weeks	13 Weeks	25 Weeks	53 Weeks	13 Weeks	53 Weeks
Males							
0	7.30±1.32	8.38±1.61	9.08±1.50	9.95±1.04	10.20±1.15	117	130
200	7.35±0.77	8.40±0.88	9.38±1.21	9.93±1.65	10.25±1.65	123 (105)	135 (104)
1000	7.65±1.23	9.15±0.97	10.20±1.21	11.00±1.41	10.70±1.25	136 (101)	148 (113)
5000	7.25±1.34	7.90±1.93	8.23±2.05	9.00±2.25	9.13±2.29	109 (93)	121 (90)
Females							
0	6.68±0.78	7.88±0.56	8.45±0.77	8.98±0.77	9.00±0.50	132	134
200	6.85±0.78	8.05±1.24	8.78±1.36	9.65±1.46	9.85±1.74	126 (95)	143 (106)
1000	6.38±0.54	7.15±0.71	7.55±0.87	8.15±0.89	8.18±0.84	130 (98)	129 (96)
5000	6.68±0.72	7.45±1.22	7.83±1.14	7.83±1.45	7.95±1.32	110 (89)	121 (90)

*Data extracted from Study No. 97002, Tables T-4.4.1, T-4.4.2, A-4.4.1, and A-4.4.2.

Results: One male with retinal dysplasia and retinal folds in the left eye was seen at pretest; this dog was not used on study. At termination, three females showed mild inflammatory retinal scars in the nontapetal portion of the retina of one eye (two low-dose dogs and one high-dose dog). These observations were considered incidental.

2. Clinical Pathology

Blood was collected by venous puncture from all dogs at pretest, at 6 months, and prior to termination. The checked parameters (X) were examined:

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)[a]
X Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)

* = Recommended by Subdivision F (November 1984) Guidelines

[a]Only examined if signs of anemia were present

Results: All hematology parameters were similar in control and test groups at 26 and 52 weeks with the exception of platelet counts which were significantly ($p < 0.05$) decreased in the mid-dose males (26 and 52 weeks) when compared to controls. Since no similar effects were seen in high-dose males or in females at any dose, the findings were considered incidental. It was considered that there were no effects on any hematology parameters related to treatment. All values were within the normal laboratory range (data provided in report).

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
Magnesium
X Phosphorus*
X Potassium*
X Sodium*

Enzymes

X Alkaline phosphatase (ALP)
Cholinesterase
X Creatinine phosphokinase*
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other

X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
X Globulins
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
Triglycerides

* = Recommended by Subdivision F (November 1984) Guidelines

Results: Table 2 summarizes mean values of serum alkaline phosphatase (AP) activity and total bilirubin in male and female dogs at pretest, week 26, and week 52. ALP activity was significantly ($p < 0.05$) increased in high-dose males (weeks 26 and 52) and high-dose females (week 52).

Total bilirubin was increased compared to controls in high-dose males and females (week 26 and 52) and was generally increased in mid-dose females (week 26). Changes in other clinical chemistry parameters were not considered of biological importance since values were within the normal range. The other changes included a decrease in SGPT at week 26 and a decrease in albumin at week 52 in high-dose males, a decrease in total protein at week 26 in high-dose females, and a decrease in glucose in mid-dose females at week 52.

TABLE 2. Mean Clinical Chemistry Data for Dogs Fed ALANAP for 12 Months[a]

Parameter/Week	Dietary Level (ppm) 0	200	1000	5000
Serum alkaline phosphatase (IU/L)				
			Males	
Week 0	103.8±36.1[b]	87.3±35.0	86.8±13.2	86.5±31.5
Week 26	61.5±27.6	48.0±7.3	62.3±23.6	136.0*±46.5
Week 52	43.5±14.9	37.5±9.9	55.3±23.8	145.2*±52.7
			Females	
Week 0	90.5±19.7	98.0±20.2	93.0±14.6	90.0±8.5
Week 26	50.8±24.6	58.0±9.9	91.3±25.3	156.0±105.9
Week 52	41.8±16.1	39.8±6.9	81.0±13.0	147.0*±65.3
Total bilirubin (mg/dL)				
			Males	
Week 0	0.230±0.04	0.315±0.02	0.275±0.06	0.268±0.02
Week 26	0.305±0.04	0.383±0.09	0.420*±0.02	0.538*±0.03
Week 52	0.258±0.05	0.270±0.05	0.350±0.10	0.478*±0.08
			Females	
Week 0	0.120±0.03	0.118±0.02	0.150±0.07	0.158±0.01
Week 26	0.288±0.06	0.413±0.12	0.398±0.09	0.543*±0.12
Week 52	0.185±0.06	0.310±0.09	0.285±0.08	0.418*±0.05

[a]Data extracted from Study No. 97002, Tables T-4.6.2, T-4.7.2, A-4.6.2, and A-4.7.2.

[b]Mean ± standard deviation for four dogs/group.

*Signficantly different from control value, $p \leq 0.05$.

(c) Urinalysis

Urinalysis was conducted at pretest, at 26 weeks, and at 52 weeks. The checked (X) parameters were examined:

X Appearance*	X Sediment* (microscopic)	X Bilirubin*
X Volume*	X Protein*	X Blood*
X Specific gravity*	X Glucose*	Nitrate
X pH	X Ketones	X Urobilinogen

* = Recommended by Subdivision F (November 1984) Guidelines

Results: No effects on urinary parameters were noted.

3. Sacrifice and Pathology

All animals were sacrificed after 52 weeks and received a complete necropsy. The checked (X) tissues were collected for histological examination. In addition, the double-checked (XX) organs were weighed:

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	Aorta*	XX Brain
X Salivary glands*	XX Heart*	X Peripheral nerve* (sciatic nerve)
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen	X Eyes
X Jejunum*	X Thymus	
X Ileum*		
X Cecum*	Urogenital	
X Colon*		
Rectum	XX Kidneys*	Glandular
XX Liver*	X Urinary bladder*	
X Gallbladder*	XX Testes*	XX Adrenals*
X Pancreas*	X Epididymides	Lacrimal glands
	X Prostate	X Mammary glands
Respiratory	Seminal vesicle	X Thyroid*
	XX Ovaries	X Parathyroid*
X Trachea*	X Uterus	Harderian glands
X Lungs*	X Vagina	

Other

X Bone (sternum)
X Skeletal muscle*
X Skin
X All gross lesions and masses

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Organ weights

Table 3 summarizes data for absolute liver weights and liver-to-body-weight ratios. A significant ($p \leq 0.05$) increase in liver-to-body-weight ratio was observed in high-dose males; absolute mean liver weight and the liver-to-brain-weight ratio were increased 24% and 41%, respectively, over controls but the increases were not statistically significant. In high-dose females, the absolute liver weight, liver-to-body-weight ratio and liver-to-brain-weight ratio were significantly ($p \leq 0.05$) increased compared to the controls. The liver-to-body weight ratio was increased ($p \leq 0.05$) in mid-dose females, but neither the absolute liver weight nor liver-to-brain-weight ratio were significantly increased. No other organ weight changes were of toxicologic importance. Testis weight was decreased in dosed males (all groups), but values were within the normal range (historical range: 17.18 ± 3.68 g).

(b) Macroscopic

No gross findings related to compound administration were noted. Interdigital mass in the foot was seen in 0/4, 2/4, 1/4, and 3/4 males in the 0-, 200-, 1000-, and 5000-ppm groups, respectively. This was not considered related to dosing.

(c) Microscopic

No microscopic findings related to dosing were observed. The only neoplasm was a skin lipoma in a low-dose female. Hyperkeratitis of the skin on the ear was observed in one control male, one high-dose male, and one high-dose female.

Two high-dose males had cysts on the foot and one had a granuloma (corresponding to interdigital masses). Dermatitis was randomly observed in male dogs, and a mid-dose male had interstitial pneumonia. One high-dose female displayed an ovarian cyst and one mid-dose male had a pituitary cyst.

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The conduct and reporting of the study were acceptable. Summary data that were validated accurately reflected the individual animal data. The dogs may have been able to tolerate a higher dose than 5000 ppm since decreases in weight gain were minimal and no overt toxic effects were seen at the highest dose. The increases in liver weight were not accompanied by any histologic findings; however, serum alkaline phosphatase and bilirubin were increased compared to controls in both sexes in a dose-related manner with significant increases found at the highest dose tested indicating effects on the liver. The increased liver weights in females receiving 1000 ppm was marginal (11%); the liver-to-body-weight ratio was increased

25% ($p<0.05$), but the weight relative to brain weight was only increased 9% (nonsignificant, $p>0.05$). Based on effects on liver weights and the increased levels of serum alkaline phosphatase and bilirubin, the LOEL in females is assessed by the reviewers to be 1000 ppm (25.3 mg/kg/day). The LOEL in males is 5000 ppm (121 mg/kg/day).

TABLE 3. Mean Absolute Liver Weights, Liver-to Body-Weight Ratios, and Liver-to-Brain-Weight Ratios in Dogs Fed ALANAP for 12 Months[a]

Dietary Level (ppm)	Liver Weight (g ± S.D.)	Liver/Body Weight Ratio (g/kg ± S.D.)	Liver/Brain Weight Ratio (± S.D.)
		Males	
0	290.0 ± 55.8	29.02 ± 2.51	3.40 ± 0.62
200	305.0 ± 55.2	30.09 ± 2.30	3.92 ± 0.94
1000	332.5 ± 44.1	31.27 ± 2.02	4.15 ± 0.35
5000	361.3 ± 80.7	40.88 ± 2.22*	4.78 ± 1.05
		Females	
0	252.5 ± 22.2	28.09 ± 2.62	3.38 ± 0.18
200	301.3 ± 45.2	30.95 ± 2.78	3.78 ± 0.81
1000	283.8 ± 19.3	35.41 ± 3.03*	3.69 ± 0.40
5000	343.7 ± 36.4*	44.68 ± 4.83*	4.63 ± 0.88*

[a]Data extracted from Study No. 97002, Tables T-4.10.1, T-4.10.2, T-4.10.3, T-4.10.4, T-4.10.5, T-4.10.6, T-4-10.7, A-4.10.4, A-4.10.5, and A-4.10.6.

*Significantly different from controls, $p \leq 0.05$.

[redacted]

DOC930204

FINAL

009803

DATA EVALUATION REPORT

NAPTALAM

Study Type: Metabolism

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Author Karen Gan Date 10/15/92
Karen Gan, M.S.

Reviewer William L. McLellan Date 10/15/92
William McLellan, Ph.D.

QA/QC Manager Sharon A. Segal Date 10/15/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-67, 93-69
Project Officer: James Scott

00038

EPA Reviewer: Stephen C. Dapson, Ph.D.
Review Section I, Toxicology Branch II,
Health Effects Division

Signature: Stephen C. Dapson
Date: 10/20/92

EPA Section Head: Yiannakis M. Ioannou, Ph.D.
Review Section I, Toxicology Branch II,
Health Effects Division

Signature: Y. M. Ioannou
Date: 10/21/92

009803

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats

EPA IDENTIFICATION NUMBERS:

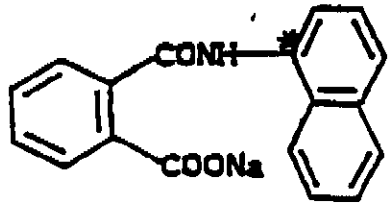
Tox. Chem. Number: 780A and 592

EPA Pesticide Chemical Code: 030703

MRID Numbers: 402745-02; 418600-03

TEST MATERIAL: Naptalam (generic), ALANAP®

SYNONYM: 2[(1-Naphthalenylamino)carbonyl]benzoic acid, sodium salt



* denotes the position of the [^{14}C] label

SPONSOR: Uniroyal Chemical Company, Inc., Bethany, CT

TESTING FACILITIES: 1) Uniroyal Chemical Company, Inc., Crop Protection Department, Chemistry Section, R&D, Naugatuck, CT; and 2) BIOTEK, Inc., Woburn, MA

AUTHORS: J.P. McManus (Uniroyal) and M.H. Gay (BIOTEK)

REPORTS: Analysis of Urine Samples from [14C] Naptalam (ALANAP) Rat Metabolism Study. MRID No. 402745-02. Appendix I contained two studies: 1) Rat Metabolism of 14C-ALANAP Pilot Study, Sponsor - Uniroyal Chemical Co., Inc., Test Facility - BIOTEK, Inc., Study No. 86038A, November 21, 1986; 2) Rat Metabolism of 14C ALANAP Final Report, Sponsor - Uniroyal Chemical Co., Inc., Test Facility - BIOTEK, Inc., Study No. 86038B, February 2, 1987.

Supplemental Report: Analysis of Percent Active Ingredient of the Test Compound. Uniroyal Project NO. 8660, BIOTEK Study No. 86038B. MRID No. 418600-03.

DATE: July 7, 1987 (Report) and April 1991 (Supplemental Report)

00039

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of naptalam were studied in groups of male and female CD rats administered a single oral dose of 250 or 1000 mg/kg [^{14}C]naptalam by gavage.

[^{14}C]Naptalam was rapidly absorbed, distributed, and excreted in rats at both dose levels. The 7-day recoveries were at least 84.85% of the administered dose for all dosing groups, with higher recoveries in the males. The elimination of radioactivity in the urine (36.39-45.30%) was almost comparable for all male and female dose groups. The radioactivity in the feces was 59.13-67.73% in the male groups and 41.56-43.10% in the female groups. The elimination data suggest that absorption of naptalam is rapid, bioaccumulation is low, and excretion occurs in the feces and urine. The authors concluded that most of the radioactivity in the feces was due to unabsorbed test material because most of the fecal elimination occurred within 24 hours. This explanation could not be confirmed because intravenous dosing was not conducted to provide further information on the fecal and urinary elimination pattern. The urine contained one major radioactive band which was identified as the unmetabolized parent compound. No metabolites were identified in the urine. Since the metabolism of naptalam was not evaluated in the feces, the complete metabolite pattern of naptalam in rats can not be determined. The study also indicates that naptalam and/or its metabolites do not bioaccumulate to an appreciable extent following oral exposure since all the tissues contained negligible levels of radioactivity at 7 days postexposure.

Based on these study results, absorption, distribution, and elimination of naptalam do not appear to be sex or dose related. No conclusion can be made regarding sex- or dose-related differences in the metabolism of naptalam since radioactivity in the feces was not analyzed. Furthermore, no metabolic pathway could be determined for naptalam. The study also showed that administration of 250 and 1000 mg/kg naptalam did not induce any apparent treatment-related clinical effects.

STUDY CLASSIFICATION: The study is classified as Supplementary. This study may be upgraded if the following additional data are provided: 1) identification of metabolites in the feces to evaluate sex-related differences in metabolism and determine metabolic pathway of naptalam; 2) intravenous dosing study to evaluate fecal elimination (i.e., unabsorbed test material and/or biliary excretion); 3) repeated dosing study to evaluate toxicokinetic differences with different dosing regimen.

A. MATERIALS

1. Test Substance

The unlabeled test material (BIOTEK, Inc., identification number 86038, lot number GMS #4785, JPM 8660) was administered by oral gavage. The 100% active ingredient was sodium N-1-naphthyl-phthalamate (Supplementary Report).

Radiolabeled naptalam (lot number 850607, JPM 8660) was labeled with a ^{14}C-label at the naphthalene ring. The mean radiochemical purity was not reported.

2. Test Animals

Male and female CD rats were obtained from Charles River Laboratories, Wilmington. MA. A single oral gavage dose of 250 or 1000 mg/kg labeled naptalam was administered to groups of 5 males and 5 females that were sacrificed at day 7 postexposure.

In a pilot study, three male rats were administered a single oral dose of 852.2 mg/kg of labeled naptalam to measure the amount of radioactivity expired as CO_2.

B. METHODS

1. Acclimation

In the primary study (Appendix I), rats were housed individually in stainless steel cages during quarantine (9 days) and acclimatized in stainless steel metabolism cages 4 days prior to exposure. Animals were provided Purina chow diet® and water ad libitum throughout the study. The authors did not report on contaminants in the food and water which may interfere with the study.

2. Dosing Solutions

The radiolabeled stock solutions (28.5 and 113.1 mg/mL) were dissolved in distilled water 16 hours before administration. The labeled preparations had specific activities of 1.39×10^6 dpm/mL for the 28.5 mg/mL solution and 1.42×10^6 dpm/mL for the 113.1 mg/mL solution. The doses were administered by gavage using a constant dosage volume of 8.8 mL/kg. Although the stability of the solutions were not reported, the test material does not appear to be volatile and is probably relatively stable.

Groups of rats (5/sex/group) were given single oral doses of 250 or 1000 mg/kg [^{14}C]naptalam. Animals were sacrificed at 7 days postexposure in a CO_2 chamber. The control group consisted of 1 male and 1 female rat receiving water. There was no indication on how the study doses were chosen. In the pilot study, the dose was reportedly reduced from 1000 mg/kg to 852.2 mg/kg to conduct the study on time (no further explanation was given for this statement).

3. Sample Collection

The urine and feces were collected from animals at the following intervals: 1, 2, 4, 8, 12, and 24 hours and 1.5, 2, 3, 4, 5, 6, and 7 days after exposure to the [^{14}C]-labeled dose of naptalam. Fecal samples and tissues were homogenized in distilled water in either a motor-driven Potter-Elvehjem tissue grinder at 2000 rpm (small samples) or a Waring blender (large samples). Homogenates and bone were oxidized in a sample oxidizer by Cambridge Analytical Laboratories (Boston, MA) and then oxidized samples were counted by BIOTEK. Blood was mixed gently with Protosol/ethanol, incubated at 60°C, then incubated with 30% H_2O_2, and shaken with HCl. Radioactivity in feces, urine, tissues, and blood samples was counted in duplicate using a Beckman Model LS 100C liquid scintillation counter. Mean counts per minute (cpm) in tissue homogenates of the control rats were used as background levels. Radioactivity of cage or urine funnel washings were not collected. Methods for statistical analyses were limited to means and standard deviations.

In the pilot study, urine, feces, and expired air as $^{14}CO_2$ were collected at 1, 2, 4, 8, 12, and 24 hours after a single oral gavage dose of 852.2 mg/kg naptalam. Expiration as CO_2 was 0.05% of the administered radioactivity after 7-days postexposure in the 100-mg/kg dosed rats. Expired CO_2 was not collected in the primary study because this was a minor elimination pathway.

4. Metabolite Analysis

In the metabolite analysis, urine samples collected at 1, 2, 4, 8, 12, and 24 hours after treatment were analyzed by high-performance liquid chromatography (HPLC). Radioactive bands were quantified by liquid scintillation spectrometry. The fecal samples, however, were not examined for metabolites.

5. Protocols

The methods followed the study protocol. Figures showing the excretion of naptalam is presented in the CBI appendix of this report (CBI Appendix pp. 38 and 39).

C. REPORTED RESULTS

1. Clinical Observations and Gross Necropsy

In the pilot study and the preliminary study (Appendix I), rats were somnolent, although they did respond when samples were collected. In both the pilot and preliminary study, piloerection was observed in all animals, including controls. The authors believed that this was probably due to the trauma of the treatment. At the 7-day sacrifice, all organs were normal except for the lungs which had hemorrhages with areas of consolidation in random locations. The authors believe that this finding is a intercurrent pulmonary disease due to variety of infectious agents, but they did not confirm this with further tests.

2. Elimination and Recovery

There was no major dose-related difference in the elimination of naptalam following a single oral dose of 250 or 1000 mg/kg naptalam. The mean total recoveries of radioactivity ranged from 84.85% to 107.54% of the administered dose at 7 days postexposure, with higher recoveries in the male groups (Table 1). Approximately 40% of the administered dose was recovered in the urines of the low- and high-dose groups. Recovery in the feces was 59.13-67.73% in the low- and high-dose males and 41.56-43.10% in the low- and high-dose females. Overall, fecal excretion peaked at 24 hours and urinary excretion peaked at 8 hours. Most of the administered radioactivity in the feces and urine was recovered by 48 and 24 hours, respectively (CBI Appendix I, Figures 1 and 2, pp. 38 and 39).

In the pilot study. the mean recoveries of three male rats was 29.97% and 25.04% of the administered radioactivity in the 24-hour urine and feces, respectively. Exhaled radioactivity as CO_2 collection was negligible (0.03%) within 24 hours.

3. Tissue Distribution

The mean radioactivities in the tissues were low in both treatment groups (0-3.52%) (Table 1). Because counts were corrected for background using a control male rat, percentage of recovery was negative in the low-dose male group (i.e., assumed 0% recovery). At 7 days postexposure, highest percentages of recovery for the high-dose males and the low- and high-dose females were in the muscle (0.79-1.22%), liver (0.32-0.42%), fat (0.15-0.16%), and kidneys (0.09-0.18%) (CBI Appendix I, Table 1, p. 37). Radioactivities in other tissues were $\leq$0.1% at 7 days postexposure.

4. Metabolism

HPLC chromatography detected only one ^{14}C-component in all urine samples that were analyzed. Comparing this radioactive component to the [^{14}C]-naptalam standard, elution occurred at 26.5 minutes for both bands. Electron ionization mass spectroscopy (MS) confirms that the radioactive component in the urine is the parent compound, naptalam, because molecular weight peak (273 m/e) and characteristic peaks were observed in the standard and the sample. Another radioactive component (143 m/e) was identified in the mass spectra as 1-naphthylamine. However, this compound was not detected in the urine samples by HPLC, suggesting that 1-naphthylamine was formed during the mass spectral analysis and is not a urinary metabolite of naptalam.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

The authors concluded that naptalam is eliminated in the urine and feces of rats. Most of the recovery of the radioactivity in the excreta occurred within 24 hours postexposure. The fecal excretion during the first 24 hours is probably unabsorbed test material (40% of administered dose) while the fecal excretion after 24 hours may include naptalam and/or its metabolite(s) that have undergone enterohepatic circulation.

Therefore, these data suggest that between 43 and 60% of the administered material was absorbed. Analysis of tissues indicates that the bone, liver, and muscle had the most significant quantities of radioactivity (i.e., test material or its metabolite(s)). Metabolite analysis indicates that the parent compound, naptalam, was eliminated virtually unmetabolized in the urine because of its polar nature.

Quality assurance statements and statements of compliance with Good Laboratory Practices for the study were signed on February 2, 1987 and January 21, 1987, respectively.

E. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study adequately described the absorption and distribution of [^{14}C]naptalam in rats following oral exposure, but did not adequately describe the metabolism and excretion of naptalam. The data indicate that labeled naptalam is rapidly and extensively absorbed from the gastrointestinal tract and eliminated to a similar extent in the feces and urine for all dosing groups. The slightly increased elimination of radioactivity in the feces of the male groups may be due to a sex-related difference in the metabolic pathway of naptalam, however, the lack of data regarding metabolites in the feces preclude a clear assessment of this hypothesis. Furthermore, intravenous dosing would provide information on whether radioactivity in the feces was due primarily to unabsorbed test material or through biliary excretion.

The low tissue levels of radioactivity, as well as the rapid elimination, at 7 days postexposure, demonstrate that bioaccumulation and retention of naptalam and/or its metabolites are low in rats. The metabolism of naptalam does not appear to be extensive following oral dosing since the only radioactive component identified in the urine was the unmetabolized parent compound. However, metabolite analysis was not conducted in the feces to fully evaluate the metabolism of naptalam.

TABLE 1 Mean Percent Recovery of Radioactivity 7 Days After Oral Administration of Naptalam to Rats

Dose Group	Sex[a]	Percent of Administered Dose Recovered			
		Urine	Feces	Tissues[b]	Total Recovery
250 mg/kg	Male	40.93	59.13	0.0[c]	100.06
	Female	40.44	41.56	2.85	84.85
1000 mg/kg	Male	36.39	67.63	3.52	107.54
	Female	45.30	43.10	2.90	91.30

[a]5 animals/sex

[b]Includes bone, blood, and muscle.

[c]The tissue counts in the control rat were used as background and resulted in mean negative percentages in most of the tissues. Therefore, the percentage of recovery was assumed to be 0%.

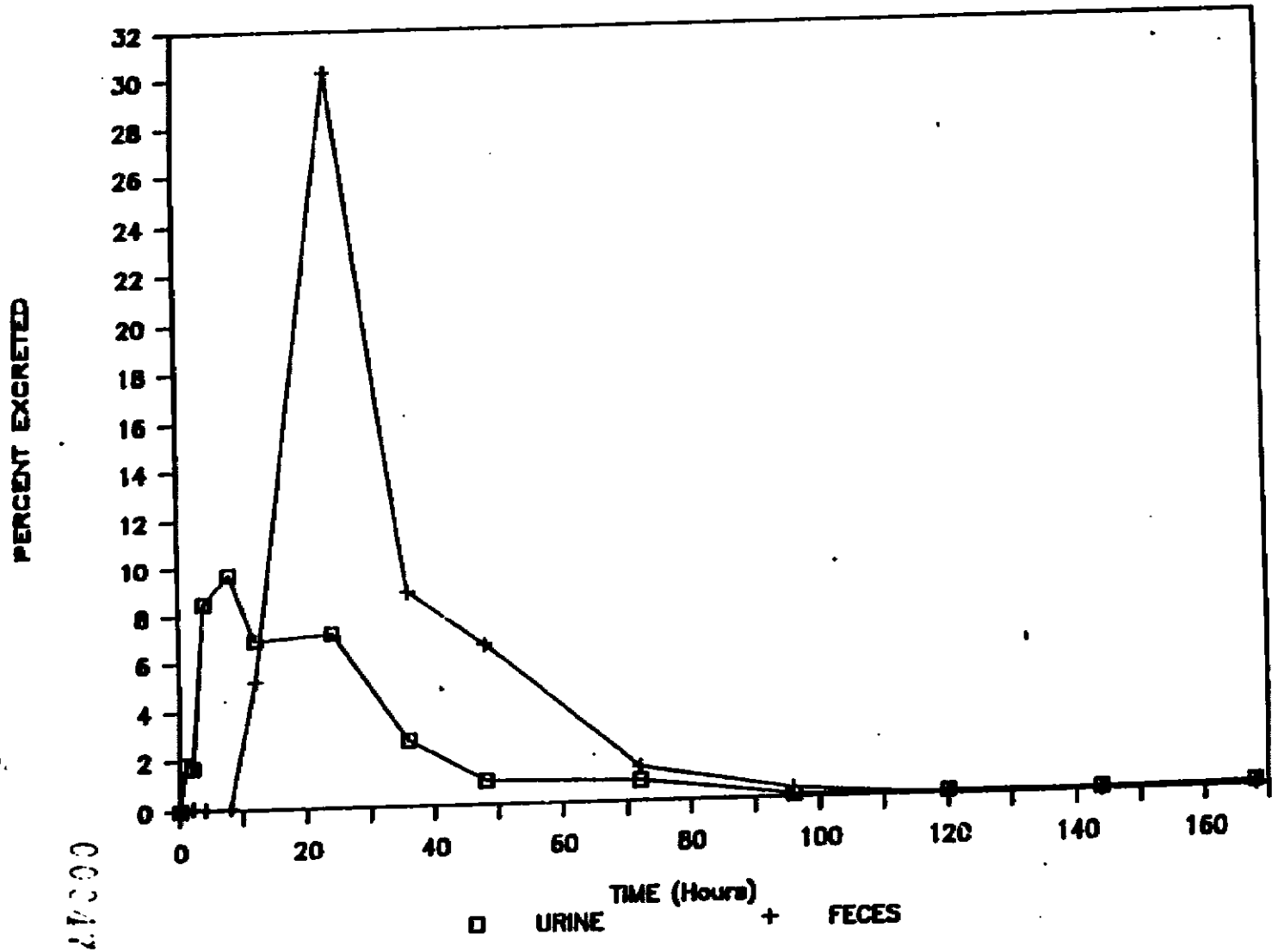
Source: CBI Table 1, p. 36

CBI APPENDIX

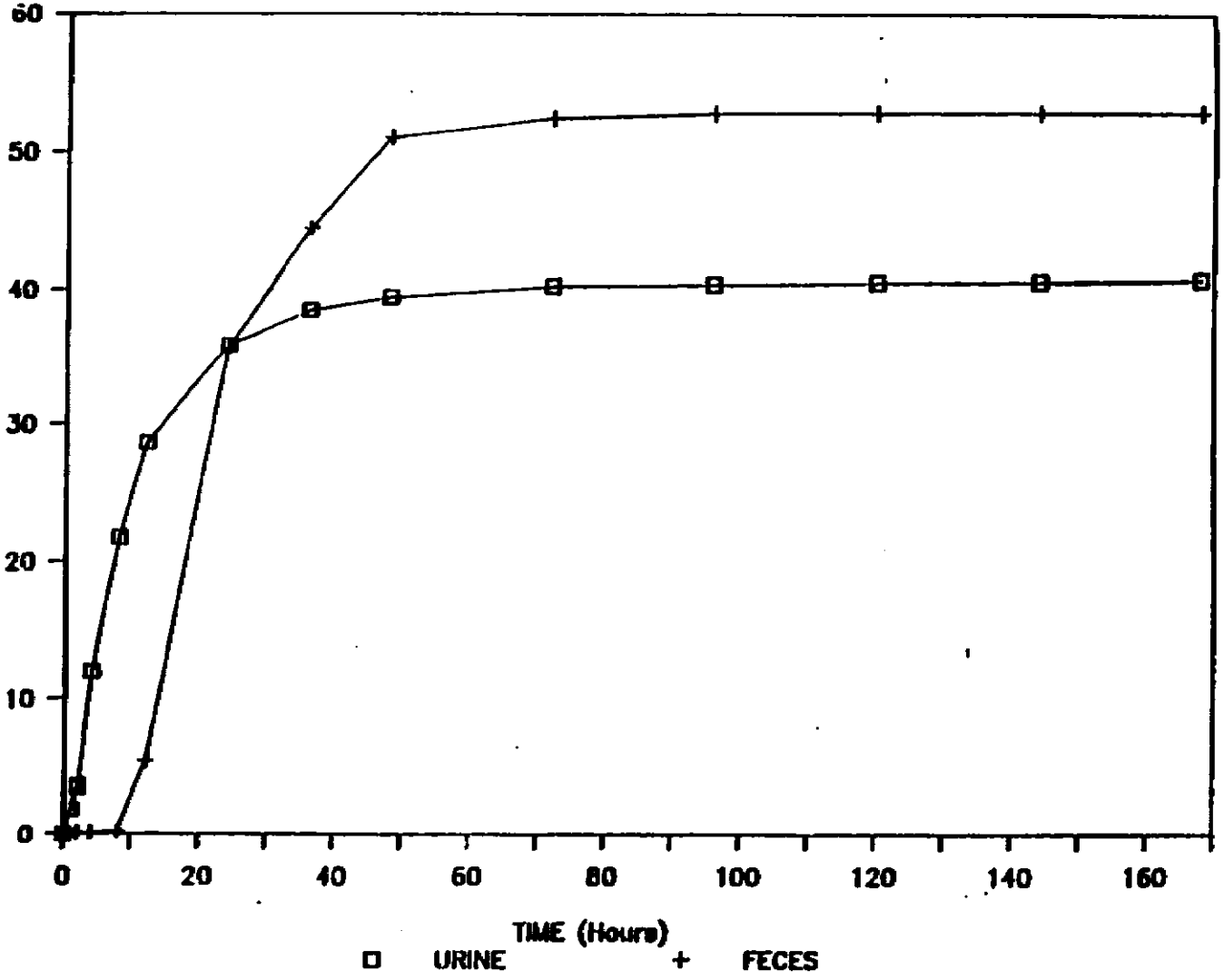
Figures on Naptalam Excretion
(CBI Appendix I, pp. 38 and 39)

FIGURE 1

ALANAP EXCRETION



RATE OF ALANAP EXCRETION



REVIEWER

[redacted]

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

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MAY 14 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of dermal sensitization study and teratology study in rabbits with Naptalam

EPA ID #400-49 & 400-345; EPA Record #170807 & 170808; EPA Accession # 261889; Caswell #592; Tox Branch Project #1720 & 1721.

TO: Robert Taylor/Vickie Walters (PM #25)
Fungicide/Herbicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/11/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 5/11/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 5/14/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Action Requested: Review acute toxicity data (dermal sensitization) to support "Warning" not "Danger" and review teratology study in rabbits.

Recommendations: The dermal sensitization study (Guideline §81-6) with Naptalam is classified as Core-Minimum Data. Under the conditions of this study, Alanap-L (Naptalam) produced dermal sensitization in the male and female guinea pig.

The teratology study in rabbits (Guideline §83-3) with Naptalam is classified as Core-Minimum Data. The No Observed Effect Level (NOEL) for Maternal Toxicity is 200 mg/kg/day. The Lowest Observed Effect Level (LOEL) for Maternal Toxicity is 650 mg/kg/day based on reduced body weight gain during the dosing period, mortality and clinical observation of reduced amount of stool. The NOEL for Developmental Toxicity is conservatively established at 200 mg/kg/day. The LOEL for Developmental Toxicity is 650 mg/kg/day based on the findings of increased numbers of litters (and fetuses)

C0049

presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae. However, the low number of dams for evaluation in this dose group may have restricted the adequate interpretation of the data for this group. The A/D ratio for this compound is 1, indicating that effects on the developing fetus may occur at doses that may be maternally toxic.

009803

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Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/4/87
Review Section V, Toxicology Branch/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch (TS-769C)

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I. Study Type: Dermal Sensitization
Guideline §81-6

Study Title: Delayed Contact Hypersensitivity Study in Guinea Pigs of Alanap-L

EPA Identification Numbers: EPA Identifying No. 400-49
EPA Record No. 170807
EPA Accession No. 261889
Shaugnessy No. 030702-5
Caswell No. 592
Tox Branch Project No. 1720
Document No.

Sponsor: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Testing Laboratory: Hill Top Research, Inc.

Study Number: Hill Top Research Project No. 85-1583-21

Study Date: December 26, 1985

Study Author: Edwin V. Buehler, Ph.D.

Test Material: ANALAP-L (also known as Naptalam)
Benzoic acid, 2-((1-naphthalenylamino)carbonyl)-
CAS # 132-66-1
Lot No. G064001

Vehicle: The test substance was used undiluted for the sensitization test.
For the primary irritation test, test substance dilutions in distilled water of 50%, 25% and 10% w/v were used.

Test Animal: Male and Female Hartley Albino Guinea Pigs
Supplier: Murphy Breeding Laboratories, Inc.
Weight 300 to 400 gms

This study was designed to evaluate the potential of Alanap-L (Naptalam) to produce delayed contact hypersensitivity in Guinea Pigs.

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II. Materials and Methods: A copy of the "purpose and general information" and "methods" section from the investigators report is appended. The following comments and highlights pertaining to the materials and methods are noted:

Test groups consisted of 10 animals per sex in the "test" group: 5 animals per sex in the control group: 2 animals per sex in the primary irritation phase.

Animals were kept under standard animals care conditions. They were quarantined for a period of at least 5 days before use. They received tap water and Purina Guinea Pig Chow ad lib.

The investigators employed the method of Buehler, 1965 and Ritz and Buehler, 1980.

The body weights of the animals at the start and completion of the test were not reported.

A Quality Assurance Statement was included.

III. Results:

A. Primary Irritation Phase (Pilot)

A mean grade of 0 was obtained at 24 and 48 hours (following a 6 hour patch application) for the undiluted, 50%, 25% and 10% w/v formulations in both male and female guinea pigs. No reaction was observed (see appended Table 1 from the investigators report).

B. Primary Challenge Phase

The control animals (distilled water) showed no evidence of dermal sensitization at 24 or 48 hours. For the test animals a mean skin score of 0.3 (1 animal with 1 and 11 with ±) was obtained at 24 hours and a score of 0.2 (9 with ±) at 48 hours. (see appended Table 2 from the investigators report). A score of 1 indicates slight confluent or moderate patchy erythema; a score of ± indicates slight patchy erythema. These scores when compared to distilled water control indicated that Alanap-L induced dermal sensitization in male and female guinea pigs.

IV. Conclusions:

Under the conditions of this study, Alanap-L produced dermal sensitization in the male and female quinea pig.

V. Core Classification: Core-Minimum Data.

Toxicity Category: Dermal Sensitizer.

Page _____ is not included in this copy.

Pages __53__ through __58__ are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__X__ Claimed Confidential by submitter upon submission to the Agency.

D098023

00582Z3

Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/4/87
Review Section V, Toxicology Branch/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-769C)

I. Study Type: Teratology - Guideline §83-3

Study Title: Teratology Study in Rabbits
(with Technical Alanap [Na Salt])

EPA Identification Numbers: EPA Identifying No. 400-345
EPA Record No. 170808
EPA Accession No. 261889
Shaugnessy No. 030702-5
Caswell No. 592
Tox Branch Project No. 1721
Document No.

Sponsor: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Testing Laboratory: International Research and Development Corporation
Mattawan, Michigan 49071

Study Number: IRDC Report No. 399-053

Study Date: May 31, 1985

Study Authors: Karen S. Arnold, B.S.
James L. Schardein, M.S.
Malcolm Blair, Ph.D.

Test Material: Technical Grade Alanap (as sodium salt)
(also known as Naptalam)
Benzoic acid, 2-((1-naphthalenylamino)carbonyl)-
monosodium salt
CAS # 132-67-2
Lot #3199300
Purity was not provided.

Vehicle: Deionized water.

Dosage: 0 (control), 50, 200 and 650 mg/kg/day. Test compound prepared daily as a suspension with a dosage volume of 3 ml/kg (based on most recent body weight). Administered by gavage as a single daily dose from Gestation Days 7 through 19. Dosing was conducted 3.25 to 7.5 hours into light cycle.

00359

Test Animal: Female Dutch Belted Rabbits
Received from Langshaw Farms, Augusta, Michigan
80 animals were used, received 11/1/84
Age = 4 to 4 1/2 months old

This study was designed to evaluate the developmental toxicity potential of Technical Alanap (Na salt).

II. Materials and Methods: A copy of the "Introduction" and "Methods and Procedures" is appended. The following comments and highlights pertaining to the materials and methods are noted:

Animals were kept under standard animal care procedures. All conditions were described in detail (see appended "Methods and Procedures").

Animals were treated by the supplier (Langshaw Farms) with 0.0032% sulfaquinoxaline one week prior to shipment to the investigators "...for control of cocciodosis".

Animals were quarantined for 27 days prior to use. During this period they were "...carefuly observed for changes in appearance and behavior. Animals were 5 to 5 1/2 months old prior to insemination and weighed between 2580 and 3320 gm on Gestation Day 0. They were superovulated 3 weeks prior to insemination with human chorionic gonadotropin (50 USP).

Animals were randomly assigned to study groups using a computer generated system.

The investigators employed 8 "proven" male rabbits as sperm donors. Description of the insemination procedure is appended in "Methods and Procedures". The authors stated that "Semen from one male was used to inseminate an equal number of females in each group". However, there are no data to substantiate their statement. The day of insemination was considered as Gestation Day 0.

Alanap was administered at dosage levels (as a suspension) of either 0, 50, 200 and 650 mg/kg/day by gavage as a single daily dose on Gestation Days 7 through 19.

The animals were observed twice daily prior to treatment for mortality and "overt changes in appearance and behavior". During the treatment period they were observed twice daily for mortality and once daily for "clinical signs of toxicity". Animals which died early, aborted or delivered prematurely were subjected to a post-mortem examination. The intact fetuses were examined and preserved for possible future evaluation as were the maternal tissues from the post-mortem examinations. No food consumption data were provided.

Individual maternal body weights were taken on Gestation Days 0, 7, 13, 20, 24 and 28. On Gestation Day 28 all surviving dams were sacrificed by injection of sodium pentobarbital into the marginal ear vein. They were then subjected to a post-mortem examination (maternal tissues were again preserved).

All fetuses were weighed, sexed and examined for external gross anomalies. They were then dissected, internally sexed and examined for visceral anomalies. The brain was examined by a mid coronal slice and the heart by a modified Staples method. All fetuses were then cleared and stained by a method similar to Dawson for skeletal examinations.

Statistical methodology was provided (see attached "Methods and Procedures").

A Quality Assurance Statement was provided.

III. Results

A. Maternal Clinical Observations

The investigators provided group mean and individual dam data. Table I presents the clinical observations.

TABLE I: Maternal Clinical Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# Dams	16	16	16	16
# Aborted	-	-	1	1
# Died	-	1	-	4
Nasal Discharge	3(6)[†]	-	-	2(5)
Ocular Discharge	-	-	1(5)	1(3)
Stained Haircoat	2(7)	1(3)	4(16)	3(15)
Hair Loss	1(5)	1(4)	-	-
Reduced Activity	-	-	-	2(2)
Gasping	-	-	-	1(1)
Ataxic	-	-	-	1(1)
Emaciated Appearance	-	1(8)	1(3)	2(4)
Sore in mouth	-	-	1(14)	-
Subcutaneous Mass in mouth	-	-	1(8)	-
Diarrhea	1(2)	-	2(2)	-
Soft Stool	-	1(1)	2(4)	1(4)
Reduced Amount of Stool	1(2)	1(10)	2(10)	7(26)
No Stool Apparent	-	-	1(3)	-
Red Fluid in Pan	-	1(3)	-	1(2)

[†] = # animals (# days observed)

[a] = Data extracted from IRDC Report No. 399-053 Table 1.

There were increased clinical observations at the high dose consisting of reduced amount of stool and an increase in the number of dams that died. Of those animals which died, the investigators found no specific cause of death. One animal had a severely congested lining of the trachea.

B. Maternal Body Weight Gain

The investigators provided group mean and individual dam data for maternal body weights and body weight gains. Table II presents the maternal body weight gain data.

TABLE II: Maternal Body Weight Gain (gm)[a]

Dose (mg/kg/day): Gestation Days	Control	50	200	650
0 - 7	163±59.1[†]	143±128.8	180±72.9	191±82.5
7 - 13	40±101.1	82±89.2	56±113.6	-173±244.0
13 - 20	93±64.7	58±122.3	-37±222.9	-16±142.0
20 - 24	-6±97.4	-15±95.1	100±191.6	103±68.0
24 - 28	-13±104.6	7±81.4	17±62.2	15±75.8
7 - 20	133±98.1	140±159.0	19±281.0	-72±254.2
0 - 28	277±139.2	268±329.8	359±150.3	313±198.1

[†] = values are mean±s.d.

[a] = Data extracted from IRDC Report No. 399-053 Table 4.

The provided data indicated that the high dose animals gained less weight during the early part of the dosing period (Gestation Days 7 through 13) as well as during the entire dosing period (Gestation Days 7 through 20), when compared to the controls. After the dosing period, the high dose animals gained more weight than the controls during the same period. This rebound phenomena is indicative of toxicity of the compound during the treatment period. No food consumption data were provided to allow further analysis of this effect.

C. Cesarean Section Observations

The investigators provided group mean and individual dam data for the measured parameters. Table III presents the results of these observations.

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TABLE III: Cesarean Section Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# Animals Used	16	16	16	16
# Died	0	1	0	4
# Gravid	0	0	0	0
# Non-Gravid	0	1	0	4
# Aborted	0	0	1	1
# Animals at C. Section	16	15	15	11
# Gravid	13	14	10	9
# Non-Gravid	3	1	5	2
Dams w/total resorptions	0	2	0	1
Dams w/viable fetuses	13	12	10	8
Mean Viable Fetuses	7.1±1.93	4.9±2.89	8.2±1.62	5.7±3.28
Mean Postimplantation Loss	0.5±0.78	1.3±2.09	0.6±0.97	1.2±2.05
Mean Implantations	7.5±1.81	6.2±2.19	8.8±1.40	6.9±2.80
Mean Corpora Lutea	10.1±1.38	9.3±1.83	11.4±3.60	10.2±4.02
Mean Preimplantation Loss (%)	25.2	32.1[†]	22.8	32.6
Mean Postimplantation Loss (%)	6.1	20.7	6.8	17.7
Mean Fetal Body Weight (gm)	31.2±4.67	35.1±4.72	31.5±2.40	34.2±3.78
Fetal Sex Distribution (m/f)	46/46	31/38	40/42	30/21

[†] = two animals were not included due to regressing corpora lutea
[a] = Data extracted from IRDC Report No. 399-053 Table 6.

No treatment related effects on the cesarean section parameters was noted. It also must be noted that the low dose group had a smaller mean litter size when compared to other study groups which may result from a lower number of corpora lutea and high preimplantation loss noted in this group.

D. Fetal Anomaly Observations

The investigators provided group totals and individual animal data as well as historical control data.

1. External and Visceral Examinations

The investigators did not separate observations into external and internal findings. These findings are summarized in the following Table IV. Apparently, no treatment related effects were evident.

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TABLE IV: Visceral Anomaly Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# litters examined	13	12	10	8
# fetuses examined	92	69	82	51
Craniorachischisis	1(1)/1(8)[†,††]			
Ablepharia	1(1)/1(8)[††]			
Microphthalmia	1(1)/1(8)[††]			
Cleft palate	1(1)/1(8)[††]			
Omphalocele				1(1)/2(13)
Retroesophageal aortic arch				1(1)/2(13)
Ectromelia	1(1)/1(8)			
"Tail anomaly"			1(1)/1(10)	
"Major vessel variation"[†††]	10(3)/11(23)	4(3)/6(25)	4(3)/5(30)	1(1)/2(13)
Renal pelvis not developed	1(1)/1(8)			
Hemorrhagic thymus			1(1)/1(10)	

† = #fetuses(#litters)/%fetuses(%litters)
†† = observations in the same fetus
††† = specific findings were included in individual animal data.
[a] = Data extracted from IRDC Report No. 399-053 Tables 9 and 11.

2. Skeletal Examinations

Table V presents the skeletal anomaly observations.

TABLE V: Skeletal Anomaly Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# litters examined	13	12	10	8
# fetuses examined	92	69	82	51
Craniorachischisis[†]	1(1)/1(8)[††]			
"Tail anomaly"[†]			1(1)/1(10)	
Forked Scapula	3(3)/3(23)	3(3)/4(25)	2(1)/2(10)	4(3)/8(38)
27 presacral vertebrae	14(6)/15(46)	9(5)/13(42)	9(4)/11(40)	2(2)/4(25)
13th rudimentary rib(s)	17(10)/19(77)	14(9)/20(75)	12(4)/15(40)	9(5)/18(63)
12 full pairs of ribs	13(5)/14(39)	11(5)/16(42)	15(4)/18(40)	3(2)/6(25)
Accessory skull bones	2(2)/2(15)	2(2)/3(17)	2(2)/2(20)	1(1)/2(13)
Hyoid arch(es) bent	3(3)/3(16)	6(5)/9(42)	4(2)/5(20)	6(5)/12(63)
Hyoid body unossified		1(1)/2(8)	1(1)/1(10)	
Skull, reduced ossification	5(2)/5(15)	2(2)/3(17)		
Sternebrae #5 unossified	9(5)/10(37)	3(2)/4(17)	6(4)/7(40)	4(3)/8(38)
Misaligned sternebrae	2(2)/2(15)			2(2)/4(25)
Fused sternebrae		1(1)/2(8)	1(1)/1(10)	1(1)/2(13)
Vertebral variation				1(1)/2(13)
Carpal flexure	1(1)/1(8)			

† = noted in visceral examinations
†† = #fetuses(#litters)/%fetuses(%litters)
[a] = Data extracted from IRDC Report No. 399-053 Table 11.

The utility of the high dose is restricted due to the small number of litters (8) as compared to the control group (13). However, there may be an indication of developmental toxicity in the high dose as noted by an increase in the number of litters (and fetuses) presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae.

IV. Conclusions:

The No Observed Effect Level (NOEL) for Maternal Toxicity is 200 mg/kg/day. The Lowest Observed Effect Level (LOEL) for Maternal Toxicity is 650 mg/kg/day based on reduced body weight gain during the dosing period, mortality and clinical observation of reduced amount of stool.

The NOEL for Developmental Toxicity is conservatively established at 200 mg/kg/day. The LOEL for Developmental Toxicity is 650 mg/kg/day based on the findings of increased numbers of litters (and fetuses) presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae. However, the low number of dams for evaluation in this dose group may have restricted the adequate interpretation of the data for this group.

The A/D ratio for this compound is 1, indicating that effects on the developing fetus may occur at doses that may be maternally toxic.

V. Core Classification: Core-Minimum Data.

Maternal Toxicity NOEL = 200 mg/kg/day
Maternal Toxicity LOEL = 650 mg/kg/day
Developmental Toxicity NOEL = 200 mg/kg/day
Developmental Toxicity LOEL = 650 mg/kg/day
A/D Ratio = 1

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Pages __66__ through __73__ are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

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__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__X__ Claimed Confidential by submitter upon submission to the Agency.

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

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MAY 14 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of dermal sensitization study and teratology study in rabbits with Naptalam

EPA ID #400-49 & 400-345; EPA Record #170807 & 170808; EPA Accession # 261889; Caswell #592; Tox Branch Project #1720 & 1721.

TO: Robert Taylor/Vickie Walters (PM #25)
Fungicide/Herbicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/11/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 5/11/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 5/14/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Action Requested: Review acute toxicity data (dermal sensitization) to support "Warning" not "Danger" and review teratology study in rabbits.

Recommendations: The dermal sensitization study (Guideline §81-6) with Naptalam is classified as Core-Minimum Data. Under the conditions of this study, Alanap-L (Naptalam) produced dermal sensitization in the male and female guinea pig.

The teratology study in rabbits (Guideline §83-3) with Naptalam is classified as Core-Minimum Data. The No Observed Effect Level (NOEL) for Maternal Toxicity is 200 mg/kg/day. The Lowest Observed Effect Level (LOEL) for Maternal Toxicity is 650 mg/kg/day based on reduced body weight gain during the dosing period, mortality and clinical observation of reduced amount of stool. The NOEL for Developmental Toxicity is conservatively established at 200 mg/kg/day. The LOEL for Developmental Toxicity is 650 mg/kg/day based on the findings of increased numbers of litters (and fetuses)

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presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae. However, the low number of dams for evaluation in this dose group may have restricted the adequate interpretation of the data for this group. The A/D ratio for this compound is 1, indicating that effects on the developing fetus may occur at doses that may be maternally toxic.

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Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/4/87
Review Section V, Toxicology Branch/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch (TS-769C)

I. Study Type: Dermal Sensitization
Guideline §81-6

Study Title: Delayed Contact Hypersensitivity Study in Guinea Pigs of Alanap-L

EPA Identification Numbers: EPA Identifying No. 400-49
EPA Record No. 170807
EPA Accession No. 261889
Shaugnessy No. 030702-5
Caswell No. 592
Tox Branch Project No. 1720
Document No.

Sponsor: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Testing Laboratory: Hill Top Research, Inc.

Study Number: Hill Top Research Project No. 85-1583-21

Study Date: December 26, 1985

Study Author: Edwin V. Buehler, Ph.D.

Test Material: ANALAP-L (also known as Naptalam)
Benzoic acid, 2-((1-naphthalenylamino)carbonyl)-
CAS # 132-66-1
Lot No. G064001

Vehicle: The test substance was used undiluted for the sensitization test.
For the primary irritation test, test substance dilutions in distilled water of 50%, 25% and 10% w/v were used.

Test Animal: Male and Female Hartley Albino Guinea Pigs
Supplier: Murphy Breeding Laboratories, Inc.
Weight 300 to 400 gms

This study was designed to evaluate the potential of Alanap-L (Naptalam) to produce delayed contact hypersensitivity in Guinea Pigs.

II. Materials and Methods: A copy of the "purpose and general information" and "methods" section from the investigators report is appended. The following comments and highlights pertaining to the materials and methods are noted:

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Test groups consisted of 10 animals per sex in the "test" group: 5 animals per sex in the control group: 2 animals per sex in the primary irritation phase.

Animals were kept under standard animals care conditions. They were quarantined for a period of at least 5 days before use. They received tap water and Purina Guinea Pig Chow ad lib.

The investigators employed the method of Buehler, 1965 and Ritz and Buehler, 1980.

The body weights of the animals at the start and completion of the test were not reported.

A Quality Assurance Statement was included.

III. Results:

A. Primary Irritation Phase (Pilot)

A mean grade of 0 was obtained at 24 and 48 hours (following a 6 hour patch application) for the undiluted, 50%, 25% and 10% w/v formulations in both male and female guinea pigs. No reaction was observed (see appended Table 1 from the investigators report).

B. Primary Challenge Phase

The control animals (distilled water) showed no evidence of dermal sensitization at 24 or 48 hours. For the test animals a mean skin score of 0.3 (1 animal with 1 and 11 with ±) was obtained at 24 hours and a score of 0.2 (9 with ±) at 48 hours. (see appended Table 2 from the investigators report). A score of 1 indicates slight confluent or moderate patchy erythema: a score of ± indicates slight patchy erythema. These scores when compared to distilled water control indicated that Alanap-L induced dermal sensitization in male and female guinea pigs.

IV. Conclusions:

Under the conditions of this study, Alanap-L produced dermal sensitization in the male and female quinea pig.

V. Core Classification: Core-Minimum Data.

Toxicity Category: Dermal Sensitizer.

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The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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__X__ FIFRA registration data.

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_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__X__ Claimed Confidential by submitter upon submission to the Agency.

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Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/4/87
Review Section V, Toxicology Branch/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-769C)

I. Study Type: Teratology - Guideline §83-3

Study Title: Teratology Study in Rabbits
(with Technical Alanap [Na Salt])

EPA Identification Numbers: EPA Identifying No. 400-345
EPA Record No. 170808
EPA Accession No. 261889
Shaugnessy No. 030702-5
Caswell No. 592
Tox Branch Project No. 1721
Document No.

Sponsor: Uniroyal, Inc.
74 Amity Road
Bethany, CT 06525

Testing Laboratory: International Research and Development Corporation
Mattawan, Michigan 49071

Study Number: IRDC Report No. 399-053

Study Date: May 31, 1985

Study Authors: Karen S. Arnold, B.S.
James L. Schardein, M.S.
Malcolm Blair, Ph.D.

Test Material: Technical Grade Alanap (as sodium salt)
(also known as Naptalam)
Benzoic acid, 2-((1-naphthalenylamino)carbonyl)-
monosodium salt
CAS # 132-67-2
Lot #3199300
Purity was not provided.

Vehicle: Deionized water.

Dosage: 0 (control), 50, 200 and 650 mg/kg/day. Test compound prepared daily as a suspension with a dosage volume of 3 ml/kg (based on most recent body weight). Administered by gavage as a single daily dose from Gestation Days 7 through 19. Dosing was conducted 3.25 to 7.5 hours into light cycle.

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Test Animal: Female Dutch Belted Rabbits
Received from Langshaw Farms, Augusta, Michigan
80 animals were used, received 11/1/84
Age = 4 to 4 1/2 months old

This study was designed to evaluate the developmental toxicity potential of Technical Alanap (Na salt).

II. Materials and Methods: A copy of the "Introduction" and "Methods and Procedures" is appended. The following comments and highlights pertaining to the materials and methods are noted:

Animals were kept under standard animal care procedures. All conditions were described in detail (see appended "Methods and Procedures").

Animals were treated by the supplier (Langshaw Farms) with 0.0032% sulfaquinoxaline one week prior to shipment to the investigators "...for control of cocciodosis".

Animals were quarantined for 27 days prior to use. During this period they were "...carefuly observed for changes in appearance and behavior. Animals were 5 to 5 1/2 months old prior to insemination and weighed between 2580 and 3320 gm on Gestation Day 0. They were superovulated 3 weeks prior to insemination with human chorionic gonadotropin (50 USP).

Animals were randomly assigned to study groups using a computer generated system.

The investigators employed 8 "proven" male rabbits as sperm donors. Description of the insemination procedure is appended in "Methods and Procedures". The authors stated that "Semen from one male was used to inseminate an equal number of females in each group". However, there are no data to substantiate their statement. The day of insemination was considered as Gestation Day 0.

Alanap was administered at dosage levels (as a suspension) of either 0, 50, 200 and 650 mg/kg/day by gavage as a single daily dose on Gestation Days 7 through 19.

The animals were observed twice daily prior to treatment for mortality and "overt changes in appearance and behavior". During the treatment period they were observed twice daily for mortality and once daily for "clinical signs of toxicity". Animals which died early, aborted or delivered prematurely were subjected to a post-mortem examination. The intact fetuses were examined and preserved for possible future evaluation as were the maternal tissues from the post-mortem examinations. No food consumption data were provided.

Individual maternal body weights were taken on Gestation Days 0, 7, 13, 20, 24 and 28. On Gestation Day 28 all surviving dams were sacrificed by injection of sodium pentobarbital into the marginal ear vein. They were then subjected to a post-mortem examination (maternal tissues were again preserved).

All fetuses were weighed, sexed and examined for external gross anomalies. They were then dissected, internally sexed and examined for visceral anomalies. The brain was examined by a mid coronal slice and the heart by a modified Staples method. All fetuses were then cleared and stained by a method similar to Dawson for skeletal examinations.

Statistical methodology was provided (see attached "Methods and Procedures").

A Quality Assurance Statement was provided.

III. Results

A. Maternal Clinical Observations

The investigators provided group mean and individual dam data. Table I presents the clinical observations.

TABLE I: Maternal Clinical Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# Dams	16	16	16	16
# Aborted	-	-	1	1
# Died	-	1	-	4
Nasal Discharge	3(6)[†]	-	-	2(5)
Ocular Discharge	-	-	1(5)	1(3)
Stained Haircoat	2(7)	1(3)	4(16)	3(15)
Hair Loss	1(5)	1(4)	-	-
Reduced Activity	-	-	-	2(2)
Gasping	-	-	-	1(1)
Ataxic	-	-	-	1(1)
Emaciated Appearance	-	1(8)	1(3)	2(4)
Sore in mouth	-	-	1(14)	-
Subcutaneous Mass in mouth	-	-	1(8)	-
Diarrhea	1(2)	-	2(2)	-
Soft Stool	-	1(1)	2(4)	1(4)
Reduced Amount of Stool	1(2)	1(10)	2(10)	7(26)
No Stool Apparent	-	-	1(3)	-
Red Fluid in Pan	-	1(3)	-	1(2)

† = # animals (# days observed)

a = Data extracted from IRDC Report No. 399-053 Table 1.

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There were increased clinical observations at the high dose consisting of reduced amount of stool and an increase in the number of dams that died. Of those animals which died, the investigators found no specific cause of death. One animal had a severely congested lining of the trachea.

B. Maternal Body Weight Gain

The investigators provided group mean and individual dam data for maternal body weights and body weight gains. Table II presents the maternal body weight gain data.

TABLE II: Maternal Body Weight Gain (gm)[a]

Dose (mg/kg/day): Gestation Days	Control	50	200	650
0 - 7	163±59.1†	143±128.8	180±72.9	191±82.5
7 - 13	40±101.1	82±89.2	56±113.6	-173±244.0
13 - 20	93±64.7	58±122.3	-37±222.9	-16±142.0
20 - 24	-6±97.4	-15±95.1	100±191.6	103±68.0
24 - 28	-13±104.6	7±81.4	17±62.2	15±75.8
7 - 20	133±98.1	140±159.0	19±281.0	-72±254.2
0 - 28	277±139.2	268±329.8	359±150.3	313±198.1

† = values are mean±s.d.

[a] = Data extracted from IRDC Report No. 399-053 Table 4.

The provided data indicated that the high dose animals gained less weight during the early part of the dosing period (Gestation Days 7 through 13) as well as during the entire dosing period (Gestation Days 7 through 20), when compared to the controls. After the dosing period, the high dose animals gained more weight than the controls during the same period. This rebound phenomena is indicative of toxicity of the compound during the treatment period. No food consumption data were provided to allow further analysis of this effect.

C. Cesarean Section Observations

The investigators provided group mean and individual dam data for the measured parameters. Table III presents the results of these observations.

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TABLE III: Cesarean Section Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# Animals Used	16	16	16	16
# Died	0	1	0	4
# Gravid	0	0	0	0
# Non-Gravid	0	1	0	4
# Aborted	0	0	1	1
# Animals at C. Section	16	15	15	11
# Gravid	13	14	10	9
# Non-Gravid	3	1	5	2
Dams w/total resorptions	0	2	0	1
Dams w/viable fetuses	13	12	10	8
Mean Viable Fetuses	7.1±1.93	4.9±2.89	8.2±1.62	5.7±3.28
Mean Postimplantation Loss	0.5±0.78	1.3±2.09	0.6±0.97	1.2±2.05
Mean Implantations	7.5±1.81	6.2±2.19	8.8±1.40	6.9±2.80
Mean Corpora Lutea	10.1±1.38	9.3±1.83	11.4±3.60	10.2±4.02
Mean Preimplantation Loss (%)	25.2	32.1[†]	22.8	32.6
Mean Postimplantation Loss (%)	6.1	20.7	6.8	17.7
Mean Fetal Body Weight (gm)	31.2±4.67	35.1±4.72	31.5±2.40	34.2±3.78
Fetal Sex Distribution (m/f)	46/46	31/38	40/42	30/21

[†] = two animals were not included due to regressing corpora lutea
[a] = Data extracted from IRDC Report No. 399-053 Table 6.

No treatment related effects on the cesarean section parameters was noted. It also must be noted that the low dose group had a smaller mean litter size when compared to other study groups which may result from a lower number of corpora lutea and high preimplantation loss noted in this group.

D. Fetal Anomaly Observations

The investigators provided group totals and individual animal data as well as historical control data.

1. External and Visceral Examinations

The investigators did not separate observations into external and internal findings. These findings are summarized in the following Table IV. Apparently, no treatment related effects were evident.

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TABLE IV: Visceral Anomaly Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# litters examined	13	12	10	8
# fetuses examined	92	69	82	51
Craniorachischisis	1(1)/1(8)[†,††]			
Ablepharia	1(1)/1(8)[††]			
Microphthalmia	1(1)/1(8)[††]			
Cleft palate	1(1)/1(8)[††]			
Omphalocele				1(1)/2(13)
Retroesophageal aortic arch				1(1)/2(13)
Ectromelia	1(1)/1(8)			
"Tail anomaly"			1(1)/1(10)	
"Major vessel variation"[†††]	10(3)/11(23)	4(3)/6(25)	4(3)/5(30)	1(1)/2(13)
Renal pelvis not developed	1(1)/1(8)			
Hemorrhagic thymus			1(1)/1(10)	

† = #fetuses(#litters)/%fetuses(%litters)
†† = observations in the same fetus
††† = specific findings were included in individual animal data.
[a] = Data extracted from IRDC Report No. 399-053 Tables 9 and 11.

2. Skeletal Examinations

Table V presents the skeletal anomaly observations.

TABLE V: Skeletal Anomaly Observations[a]

Dose (mg/kg/day):	Control	50	200	650
# litters examined	13	12	10	8
# fetuses examined	92	69	82	51
Craniorachischisis[†]	1(1)/1(8)[††]			
"Tail anomaly"[†]			1(1)/1(10)	
Forked Scapula	3(3)/3(23)	3(3)/4(25)	2(1)/2(10)	4(3)/8(38)
27 presacral vertebrae	14(6)/15(46)	9(5)/13(42)	9(4)/11(40)	2(2)/4(25)
13th rudimentary rib(s)	17(10)/19(77)	14(9)/20(75)	12(4)/15(40)	9(5)/18(63)
12 full pairs of ribs	13(5)/14(39)	11(5)/16(42)	15(4)/18(40)	3(2)/6(25)
Accessory skull bones	2(2)/2(15)	2(2)/3(17)	2(2)/2(20)	1(1)/2(13)
Hyoid arch(es) bent	3(3)/3(16)	6(5)/9(42)	4(2)/5(20)	6(5)/12(63)
Hyoid body unossified		1(1)/2(8)	1(1)/1(10)	
Skull, reduced ossification	5(2)/5(15)	2(2)/3(17)		
Sternebrae #5 unossified	9(5)/10(37)	3(2)/4(17)	6(4)/7(40)	4(3)/8(38)
Misaligned sternebrae	2(2)/2(15)			2(2)/4(25)
Fused sternebrae		1(1)/2(8)	1(1)/1(10)	1(1)/2(13)
Vertebral variation				1(1)/2(13)
Carpal flexure	1(1)/1(8)			

† = noted in visceral examinations
†† = #fetuses(#litters)/%fetuses(%litters)
[a] = Data extracted from IRDC Report No. 399-053 Table 11.

The utility of the high dose is restricted due to the small number of litters (8) as compared to the control group (13). However, there may be an indication of developmental toxicity in the high dose as noted by an increase in the number of litters (and fetuses) presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae.

IV. Conclusions:

The No Observed Effect Level (NOEL) for Maternal Toxicity is 200 mg/kg/day. The Lowest Observed Effect Level (LOEL) for Maternal Toxicity is 650 mg/kg/day based on reduced body weight gain during the dosing period, mortality and clinical observation of reduced amount of stool.

The NOEL for Developmental Toxicity is conservatively established at 200 mg/kg/day. The LOEL for Developmental Toxicity is 650 mg/kg/day based on the findings of increased numbers of litters (and fetuses) presenting with forked scapula, hyoid arch(es) bent and misaligned sternebrae. However, the low number of dams for evaluation in this dose group may have restricted the adequate interpretation of the data for this group.

The A/D ratio for this compound is 1, indicating that effects on the developing fetus may occur at doses that may be maternally toxic.

V. Core Classification: Core-Minimum Data.

Maternal Toxicity NOEL = 200 mg/kg/day
Maternal Toxicity LOEL = 650 mg/kg/day
Developmental Toxicity NOEL = 200 mg/kg/day
Developmental Toxicity LOEL = 650 mg/kg/day
A/D Ratio = 1

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Pages __91__ through __98__ are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__X__ Claimed Confidential by submitter upon submission to the Agency.

[redacted]

DOC930163

FINAL

009803

DATA EVALUATION REPORT

NAPTALAM

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll Date 9/23

Nancy E. McCarroll, B.S

Independent Reviewer Lynne Haber Date 9/23/92

Lynne Haber, Ph.D.

QA/QC Manager Sharon Segal Date 9/23/92

Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-63
Project Officer: James Scott

00039

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Stephen C. Dapson, Ph.D. Signature: Stephen C. Dapson
EPA Review Section (I), Date: 9/29/92
Toxicology Branch II/HED (H-7509C)
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Y.M. Ioannou
EPA Review Section (I), Date: 10/20/92
Toxicology Branch II/HED (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT)

EPA IDENTIFICATION Numbers:

EPA Pesticide Chemical Code: 030703
Tox Chem. Number: 780A and 592

MRID Number: 400691-03

TEST MATERIAL: Sodium alanap

SYNONYM: Naptalam

SPONSOR: Uniroyal Crop Protection, Bethany, CT

STUDY NUMBER: 8600 15-30

TESTING FACILITY: American Biogenics Corp., Woburn, MA

TITLE OF REPORT: CHO/HGPRT *In vitro* Mammalian Cell Mutation Assay on Sodium Alanap

AUTHOR: K.S. Loveday

REPORT ISSUED: January 5, 1987

CONCLUSIONS--EXECUTIVE SUMMARY: Sodium alanap at nonactivated doses ranging from 100-1500 µg/mL and S9-activated doses of 14.9-996 µg/mL did not induce a mutagenic response in CHO cells at the HGPRT locus. The study was, however, seriously compromised because of the marked differences in the cytotoxicity data obtained from the preliminary and mutational assays. For example, relative percent survival (RPC) was 50 and 56% after exposure to 2860 µg/mL -S9 and 1000 µg/mL +S9, respectively, in the preliminary test. By contrast, ~8% of the cells were recovered at 1500 µg/mL -S9 and <1% survived treatment with ≈1000 µg/mL -S9 in the mutation assay. Since the test material was reported to be stable, our reviewers assume that the inability to reproduce reasonably comparable cytotoxicity results probably resulted from substandard culture conditions rather than test material instability. The poor cloning efficiency of the background control cultures (~50% -S9, 44.7% +S9--preliminary cytotoxicity test; 55.5% -S9, 43.4% +S9--mutation assay) supports this assessment. In addition, the purity of the test material was not provided.

Based on these considerations, we conclude that the study is unacceptable and should be repeated.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: Sodium alanap

Description: Purple powder
Identification number: Lot/Batch number: DJS-050586
Purity: Not reported.
Receipt date: May 8, 1986
Stability: Stable at room temperature; light stable
Contaminants: None listed
Solvent used: Deionized water (DH_2O)
Other provided information: The test material was stored at room temperature. The frequency of dosing solution preparation was not reported.

2. Control Materials:

Negative: Culture medium (F12 medium containing 10% fetal calf serum and 20 mM Hepes buffer)

Solvent: DH_2O (not tested)

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in culture medium to yield a final concentration of 234 µg/mL.

Activation (concentrations, solvent): 9,10-Dimethyl-1,2-benzanthracene (DMBA) was prepared in dimethylsulfoxide to yield a final concentration of 15 µg/mL.

3. Activation: S9 derived from Sprague-Dawley (sex not specified)

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate (lot number R-218) was purchased from Microbiological Associates, Bethesda, MD.

S9 mix composition:

Component	Final Concentration
Isocitric acid	4.5 mg/mL
NADP	2.4 mg/mL
S9 fraction	20 µL/mL

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) __2 µg/mL__ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Eight doses (3, 10, 30, 110, 340, 1140, 2860, and 5720 µg/mL) were evaluated without S9 activation. The eight doses evaluated with S9 activation were 3, 10, 30, 100, 300, 1000, 2500, and 5000 µg/mL.

(b) Mutation assay:

(1) Nonactivated conditions: 15, 30, 50, 100, 150, 300, 500, 1000, and 1500 µg/mL; cells exposed to doses ≥100 µg/mL were cloned.

(2) S9-activated conditions: 14.9, 29.8, 49.8, 99.6, 149, 298, 498, 996, 1490, 2990, and 4980 µg/mL.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
__16__ hours (nonactivated) __4__ hours (activated).

(b) Cells exposed to positive controls for:
__16__ hours (nonactivated) __4__ hours (activated)

(c) Cells exposed to negative and/or solvent controls for: 16 hours (nonactivated) 4 hours (activated)

(d) After washing, cells were cultured for at least 7 days (expression period) before cell selection.

(e) After expression, $2x10^5$ cells/dish (6 dishes/flask) were cultured for at least 7 days in selection medium to determine numbers of mutants; 200 cells/dish (2 dishes/flask) were cultured for at least 7 days in nonselection medium to determine cloning efficiency.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria: The report indicated that mutation frequencies (MFs) $<20x10^{-6}$ were considered negative. No other criteria to establish assay validity or the biological significance of the findings were presented.

4. Protocol: None furnished

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Test: Representative data from the preliminary assessment of cytotoxicity are presented in Table 1. As shown, the absolute survival (cloning efficiency, CE) for the negative control cultures with and without S9 activation was unacceptably low (45%) or borderline acceptable (49%), respectively.

Findings with the test material indicated that the highest nonactivated (5720 µg/mL) and S9-activated (5000 µg/mL) doses were severely cytotoxic. Approximately 50% of the cells survived treatment with 2860 µg/mL -S9 and ~20% survived exposure to 2500 µg/mL +S9. For the remaining concentrations, relative percent survival (RPS) was ≥76% at ≤340 µg/mL -S9 and ≥56% at ≤1000 µg/mL +S9.

Based on these findings, 15-1500-µg/mL were selected for the nonactivated mutational assay and 14.9-4980-µg/mL were assayed in the presence of S9 activation.

2. Mutation Assays: As noted for the range-finding test, the absolute CEs for the negative controls were low (Table 2). The nonactivated and S9-activated test material was more cytotoxic than expected from the preliminary cytotoxicity data. For example, ≈50% RPS was reported at 2860 µg/mL -S9 in the preliminary test while only 7.7% survived treatment with the highest nonactivated dose (1500 µg/mL) in the mutation assay. Similarly, under S9-activated conditions, 56% RPS was recorded at 1000 µg/mL in the preliminary test and <1% survived

009803

TABLE 1. Representative Results of the Preliminary Cytotoxicity Test with Sodium Alanap

Substance	Dose (µg/mL)	S9 Activation	Average Number of Surviving Colonies[a]	Relative Percent Survival
Solvent/Negative Control				
Culture medium	--	-	98.4	100 (49)[b]
	--	+	89.4	100 (45)
Test Material				
Sodium alanap	1140[c]	-	91.0	92
	2860	-	49.5	50
	5720	-	0	<2
	100[d]	+	106.5	119
	300	+	57.0	64
	1000	+	50.0	56
	2500	+	17.5	20
	5000	+	0	<2

[a]Results from duplicate plates (200 cells/plate) in the treatment group and 8 plates in the negative control group; calculated by our reviewers.

[b]Values in () are absolute percent survival for the negative control.

[c]Relative percent survival for cultures treated with lower nonactivated doses (3, 10, 30, 110, and 340 µg/mL) was ≥76%.

[d]Results for lower S9-activated doses (3, 10, and 30 µg/mL) provided no clear evidence of cytotoxicity.

00104

TABLE 2. Representative Results of the Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assay with Sodium Alanap

Substance	Dose/mL	S9 Activation	Relative Percent Cloning Efficiency (After Treatment)[a]	Average Number of Mutant Colonies	Average Absolute Percent Cloning Efficiency (at Selection)[a]	Average Mutation Frequency $\times 10^{-6b}$
Negative Control						
Culture medium	--	-	100.0 (55.5)	0	0.44	<2.0
	--	+[c]	100.0 (43.4)	4	0.59	5.6
Positive Control						
Ethylmethane sulfonate	234 µg	-	83.1	136	0.41	276.4
9,10-Dimethyl-1,2-benz-anthracene	15 µg	+	11.8	282	0.46	510.9
Test Material						
Sodium alanap	1000 µg[d]	-	51.6	4	0.58	5.7
	1500 µg[e]	-	7.7	1[f]	0.44[f]	1.9[f]
	298 µg[d]	+	135.7	4	0.68	4.9
	498 µg[g]	+	83.5	6	0.74	6.7

[a]Based on the results of duplicate plates from two flasks per treatment, positive control, or nonactivated negative control groups or four flasks in the S9-activated negative/solvent control group; calculated by our reviewers. Values in () are absolute survival.

[b]Mutation Frequency (MF) = $\frac{\text{Average Number of Total Mutant Colonies}}{\text{No. of Cells Plated } (1.2\times10^6) \times \text{Cloning Efficiency}}$; calculated by our reviewers.

[c]Table 4 of the report (see CBI p. 18) listed 1% dimethyl sulfoxide as the negative/solvent control.

[d]Findings for lower doses (100, 150, 300, and 500 µg/mL -S9 and 14.9, 29.9, 49.8, 99.6, and 149 µg/mL +S9) did not suggest a mutagenic response.

[e]Highest assayed dose

[f]Following expression, no cells were recovered from one of the duplicate flasks.

[g]Higher doses (996, 1490, 2990, and 4980 µg/mL) were severely cytotoxic (i.e., <1% of the cells survived treatment).

exposure to a comparable dose (996 µg/mL) in the mutation assay. There were, however, no appreciable increases in either the number of mutant colonies or the MFs at any dose with or without S9 activation.

The study author concluded that sodium alanap was not mutagenic in the CHO/HGPRT forward gene mutation assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that while sodium alanap did not induce a mutagenic response in this test system, we have concerns regarding the lack of agreement between the preliminary and mutation assays relative to test material cytotoxicity. Similarly, the less than adequate CEs for the negative control cultures provide no confidence in the validity of the results. Under certain circumstances, studies with background CEs as low as 50% can be judged acceptable. However, the decision as to whether a study with low background CEs is acceptable relies heavily on the internal consistency of the data and the full assurance that assay conditions were optimal. Since the test material was reported to be stable at room temperature, we assume that the inability to reproduce a cytotoxic response at comparable doses was not related to the physical properties of the test material but was probably associated with substandard culture conditions. Additionally, the test material was prepared in DH_2O rather than a potentially cytotoxic solvent; it is, therefore, not unreasonable to have expected background CEs in the range of 70-100%. We also noted that Table 4 of the study report (see CBI p. 18) stated that "Percent survivors was calculated using the average number of colonies exposed to 1% DMSO +S9 as 100% survivors." The use of a solvent other than the test material solvent as the reference point for the experimental results is an unacceptable practice. It is doubtful, however, that exposure of the CHO cells to 1% DMSO contributed to the lower than expected negative control CEs; 1% DMSO is routinely used in mammalian cells assay with no adverse effects. In addition to the above technical deficiencies, there was no information on test material purity and several reporting discrepancies were found:

- The solvent control was listed as DH_2O but only data from cells grown in culture medium were presented.
- The S9-activated exposure medium was reported to be 90% serum free and alternatively as serum free.

Based on the above considerations; we conclude that the study is unacceptable.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated December 31, 1986). However, the above reporting discrepancies provides no confidence in the quality of the internal review.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations.

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Pages __108__ through __112__ are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__ _ __ Claimed Confidential by submitter upon submission to the Agency.

[redacted]

DOC930160

FINAL

009803

DATA EVALUATION REPORT

NAPTALAM

Study Type: Mutagenicity: In Vitro Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 9/23/92

Independent Reviewer Lynne T. Haber, Ph.D. Date 9/23/92

QA/QC Manager Sharon Segal, Ph.D. Date 9/23/92

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-64
Project Officer: James Scott

00113

009803

GUIDELINE §84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Stephen C. Dapson, Ph.D. Signature: Stephen C. Dapson 9/29/92
EPA Review Section (I), Date:
Toxicology Branch II/HED (H-7509C)
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Y.M. Ioannou
EPA Review Section (I), Date: 10/21/92
Toxicology Branch II/HED (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro chromosome aberration in Chinese hamster ovary (CHO) cells.

EPA IDENTIFICATION NUMBERS.:

EPA Pesticide Chemical Code: 030703

Tox Chem. Number: 780A and 592

MRID Number: 400691-04

TEST MATERIAL: Sodium alanap

SYNONYMS: Naptalam

SPONSOR: Uniroyal Crop Protection, Bethany, CT

STUDY NUMBER: 860015-20

TESTING FACILITY: American Biogenics Corp., Woburn, MA

TITLE OF REPORT: In Vitro Chromosomal Aberration Assay on Sodium Alanap

AUTHOR: K.S. Loveday

REPORT ISSUED: January 28, 1987

CONCLUSIONS-EXECUTIVE SUMMARY: Sodium alanap at 771, 1540, and 2570 µg/mL +S9 induced a dose-related clastogenic response in Chinese hamster ovary cells following a 2-hour treatment and a 17-hour recovery time. The dramatic increase in the aberration frequency at 2570 µg/mL +S9 (i.e., 83% of the cells with simple and complex aberrations) was accompanied by cytotoxicity. In the absence of S9 activation, 15% of the cells exposed to the highest scorable level (1490 µg/mL for 17 hours) had chromosome aberrations (simple and complex). The overall findings clearly indicate that the use of a prolonged cell harvest was necessary to demonstrate the clastogenesis of sodium alanap. Although a direct comparative evaluation of the nonactivated and S9-activated results is not possible because of the different treatment periods, the data provided convincing evidence that sodium alanap is clastogenic in cultured CHO cells. The study is, nevertheless, incomplete because information on the purity of the lot of the test material used in this assay was missing.

00114

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.2) for genetic effects, Category II, Structural Chromosome Aberrations but can be upgraded if test material purity information is submitted. Sodium alanap is, however, classified as positive in this in vitro mammalian cell cytogenetic assay.

A. MATERIALS:

1. Test Material: Sodium alanap

Description: Purple powder
Identification number: Lot number DJS-050586
Purity: Not reported
Receipt date: May 8, 1986
Stability: Stable at room temperature
Contaminants: None listed
Solvent used: Deionized water (DH_2O)
Other provided information: The test material was stored at room temperature and was reported to be soluble in H_2O up to 10%. Dilutions of the test material were used immediately after preparation.

2. Control Materials:

Negative: Untreated cells grown in McCoy's 5A medium supplemented with 10% fetal calf serum (FCS), 20 mM Hepes buffer, and antibiotics.

Positive:
Nonactivation: (Concentrations, solvent): Mitomycin C (MMC) was prepared in DH_2O to yield final concentrations of 1 and 5 µg/mL.

Activation: (Concentration, solvent): Cyclophosphamide (CP) was prepared in DH_2O to yield a final concentration of 50 µg/mL.

3. Activation: S9 derived from Sprague-Dawley (sex not specified)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 fraction was purchased from Microbiological Associates, Bethesda, MD and was identified as lot/batch number 296.

The composition of the S9 mix per mL of treatment medium was as follows:

NADP	2.4 mg
Isocitric acid	4.5 mg
S9	20 µL

4. Test Compound Concentration Used:

(a) Preliminary cytotoxicity assay: Not performed

(b) Cytogenetic assay: Two nonactivated and two S9-activated trials were conducted; doses were as follows:

(1) Nonactivated conditions:

Trial 1: 298, 497, 995, 1490, 2990, and 4980 µg/mL with a 10-10.5-hour cell harvest.

Trial 2: 149, 497, 995, 1490, 2990, and 4980 µg/mL with a 19-19.5-hour cell harvest.

(2) S9-Activated conditions:

Trial 1: 257, 771, 1540, 2570, and 5140 µg/mL with a 10-10.5-hour cell harvest.

Trial 2: 257, 771, 1540, 2570, and 5140 µg/mL with a 19-19.5-hour cell harvest.

5. Test Cells: The Chinese hamster ovary cells (CHO) used in this assay were obtained from Dr. Sheila Galloway, Litton Bionetics, Kensington, MD. CHO cells were initiated in McCoy's 5A medium supplemented with 10% FCS for ≈24 hours prior to use.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Yes.

Cell line or strain periodically check for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatment:

(a) Cells exposed to test compound for:
8 or 17 hours (nonactivated) 2 hours (activated)

(b) Cells exposed to positive controls for:
8 or 17 hours (nonactivated) 2 hours (activated)

(c) Cells exposed to negative and/or solvent controls for:
8 or 17 hours (nonactivated) 2 hours (activated)

2. Cytogenetic assay:

(a) Treatment: Duplicate cultures per treatment, per condition, per sampling time were seeded at 1.5×10^6 cells/flask and were exposed to the selected test material doses, or the negative or positive controls in both the presence and absence of S9 activation. All cultures were coded at the start of the assays.

In the nonactivated system, cells were dosed for 8 or 17 hour. Following exposure, cells were washed, reincubated in culture medium containing 0.26 µg/mL vinblastine sulfate for 2-2.5 hours and harvested. Under S9-activated conditions, cells were exposed as described above in serum-free culture medium for 2 hours, washed and reincubated in serum-supplemented culture medium for 8 or 17 hours. Vinblastine sulfate (0.26 µg/mL) was added 2-2.5 hours prior to cell harvest.

Metaphase cells were collected, treated with a hypotonic solution of 0.03 M KCl and 0.01M sodium citrate and fixed in methanol acetic acid (3:1). Slides were stained with 5% Giemsa.

(b) Metaphase analysis: One hundred cells (50 cells/culture) from each dose level of the test material and the negative/solvent controls at the two harvest intervals were scored for chromosome aberrations. A minimum of 5 metaphases with abnormal chromosomes were scored from the positive control groups. Mitotic indices (MIs) were also determined.

(c) Statistical methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay acceptability: The report indicated that a negative control frequency of 1-2% of the cells with chromosome aberrations was expected.

(b) Positive response: The test material was considered positive if >15% of the cells at one or more concentrations had chromosome aberrations.

C. REPORTED RESULTS:

1. Nonactivated Conditions: Doses evaluated in the absence of S9 activation ranged from 298-4980 µg/mL (10-10.5-hour cell harvest) and 149-4980 µg/mL (19-19.5-hour cell harvest). Following the normal cell harvest, no metaphase cells were recovered from cultures treated with the two highest doses (2990 and 4980 µg/mL). Accordingly, cultures treated with 298, 497, 995, and 1490 µg/mL were scored. MIs for the majority of groups, including the negative control were low (Table 1). Our reviewers also noted the occurrence of two rare complex aberrations (1 dicentric and 1 quadriradial) in the negative control group. Increases in the total number of aberrations, number of aberrations per cell, and the percentage of cells with aberrations were seen at 298 and 995 µg/mL of the test material. The assay was, therefore, repeated using a concentration range of 149-4980 µg/mL and a delayed cell harvest. In agreement with the earlier findings, 2990 and 4980 µg/mL sodium alanap were cytotoxic and no metaphase plates were available for analysis. The MI for the negative control was higher after the prolonged incubation and the results with the treated

cultures show that a dose-related decrease in the MI accompanied exposure to increasing levels of the test material. The data presented in Table 1 further show that at the highest scored dose, 15% of the cells had chromosome damage; the predominant type of aberrations was chromatid breaks. Based on this finding, the study author stated that the nonactivated test material produced a "weak positive response."

2. S9-Activated Conditions: No metaphases were recovered following either the normal or delayed harvests of cultures exposed to the highest assayed concentration (5140 µg/mL). A marked reduction in the MIs was also apparent at 2570 µg/mL following both cell harvests (Table 2). There was no clear indication of an increased incidence of chromosome damage in the experimental cultures after the normal cell harvest. However, powerful and dose-related clastogenic effects were observed when the sodium alanap-treated cultures were permitted a longer recovery time. At the high concentration (2570 µg/mL), the intensity of the response was comparable to the effect induced by the positive control (50 µg/mL CP; 19-hour harvest). The data further show that a dose-related increase in the yield of both simple and complex aberrations occurred at 771, 1540, and 2570 µg/mL.

From the overall findings, the study author concluded that sodium alanap caused chromosome aberrations in cultured CHO cells both in the presence and absence of S9 activation.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess, in agreement with the study author, that sodium alanap, induced an unequivocal clastogenic response in CHO cells. Although there was evidence of genotoxicity in the absence of S9 activation, the results from the S9-activated phase of testing were more compelling. These findings, however, cannot be directly compared because of the different treatment periods (2 hours with S9 versus -17 hours without S9) used in this assay system. We conclude, however, that sodium alanap was positive in a well-conducted series of assays but the study is incomplete because information on test material purity was not provided.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated December 8, 1986).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-11.

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.2) for genetic effects, Category II, Structural Chromosome Aberrations but can be upgraded if the missing information on test material purity is submitted. Sodium alanap is, however, classified as positive in this in vitro mammalian cell cytogenetic test system.

TABLE 1. Results of the Nonactivated In Vitro Chinese Hamster Ovary (CHO) Cell Cytogenetic Assays with Sodium Alanap

Substance	Dose (μg/mL)	Harvest Time (Hours)	Average Mitotic Index (%)[a]	No. of Cells Scored	Total Number of Aberrations[a,b]	No. of Aberrations per Cell[a,b]	% Cells with Aberrations[a,b]	Biologically Significant Aberrations No./Type[c]
Negative Control								
Culture medium	--	~10	0.6	100	4	0.04	3	2SB; 1QR; 1D
	--	~19	6.6	100	1	0.01	1	1TB
Positive Control								
Mitomycin C	5	~10	0.2	43	>105	>2.46	72[d]	11TB; 6SB; 4ID; 13TR; 3QR; 2CR; 6PU; 6GT
	1	~19	2.7	68	>113	>1.66	31[d]	13TB; 5SB; 2ID; 3TR; 1QR; 9CR; 8GT
Test Material								
Sodium alanap	289	~10	0.8	100	11	0.11	5	1TB; 9SB; 1ID
	497	~10	0.8	100	2	0.02	2	1TB; 1TR
	995	~10	7.0	100	>15	>0.15	5	1TB; 3SB; 1D; 1GT
	1490[e]	~10	0.5	100	2	0.02	2	1TB; 1SB
	149	~19	7.4	100	0	0.00	0	--
	497	~19	4.3	100	3	0.03	3	2TB; 1TR
	995	~19	2.4	100	7	0.07	6	3TB; 4SB
	1490[e]	~19	2.0	100	>29	>0.29	15	13TB; 5SB; 1TR; 1GT

[a]Average results from duplicate cultures; calculated by our reviewers.
[b]Gaps were not included in the reported aberrations.
[c]Abbreviations used:

TB = Chromatid Break
SB = Chromosome break
D = Dicentric
TR = Triradial
QR = Quadriradial
ID = Interstitial deletion
CR = Complex rearrangement
PU = Cell with at least 1 pulverized chromosome; counted as >1 aberration
GT = Cell with >10 aberrations; counted as >10 aberrations

[d]Conforms with the reporting laboratory's criterion for a positive response (i.e., >15% of the cells with aberrations).
[e]No metaphase cells were recovered from the two highest dose groups (2990 and 4980 μg/mL).

TABLE 2. Representative Results of the S9-Activated In Vitro Chinese Hamster Ovary (CHO) Cell Cytogenetic Assays with Sodium Alanap

Substance	Dose (μg/mL)	Harvest Time (Hours)	Average Mitotic Index (%)[a]	No. of Cells Scored	Total Number of Aberrations[a,b]	No. of Aberrations per Cell[a,b]	% Cells with Aberrations[a,b]	Biologically Significant Aberrations No./Type[c]
Negative Control								
Culture medium	--	~10	13.5	100	3	0.03	3	2TB; 1SB
	--	~19	12.5	100	3	0.03	3	2TB; 1SB
Positive Control								
Cyclophosphamide	50	~10	0.9	80	>56	>0.70	44[d]	10TB; 13SB; 1ID; 14TR; 4QR; 1R; 3CR; 1GT
	50	~19	3.5	29	>173	>5.97	100[d]	14TB; 22SB; 2ID; 16TR; 1QR; 2R; 5CR; 1PU; 11GT
Test Material								
Sodium alanap	257	~10	9.7	100	2	0.02	2	2SB
	771	~10	10.4	100	1	0.01	1	1SB
	1540	~10	7.4	100	11	0.11	11	4TB; 2ID; 4TR; 1QR
	2570[e]	~10	0.8	100	>16	>0.16	6	1TB; 5SB; 1GT
	257	~19	11.5	100	1	0.01	1	1SB
	771	~19	9.7	100	9	0.09	5	1TB; 7SB; 1TR
	1540	~19	8.9	100	35	0.35	18[d]	8TB; 9SB; 2ID; 8TR; 3QR; 1R; 4CR
	2570[e]	~19	2.3	59	>325	>5.51	83[d]	30TB; 18SB; 5ID; 17TR; 10QR; 6R; 9CR; 23GT

[a]Average results from duplicate cultures; calculated by our reviewers.
[b]Gaps were not included in the reported aberrations.
[c]Abbreviations used:

TB = Chromatid Break
SB = Chromosome break
TR = Triradial
QR = Quadriradial
ID = Interstitial deletion
R = Ring
CR = Complex rearrangement
PU = Cell with at least 1 pulverized chromosome; counted as >1 aberration
GT = Cell with >10 aberrations; counted as >10 aberrations

[d]Conforms with the reporting laboratory's criterion for a positive response (i.e., >15% of the cells with aberrations).
[e]No metaphase cells were recovered from the highest assayed dose group (5140 μg/mL).

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Pages __121__ through __125__ are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__ _ __ Claimed Confidential by submitter upon submission to the Agency.

[redacted]

DOC930161

FINAL

009803

DATA EVALUATION REPORT

NAPTALAM

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date 9/23/92
Nandy E. McCarroll, B.S

Independent Reviewer _____ Date 9/23/92
Lynne Haber, Ph.D.

QA/QC Manager _____ Date _____
Sharon Segal, Ph.D.

00126

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-65
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY MICRONUCLEUS

EPA Reviewer: Stephen C. Dapson, Ph.D.
EPA Review Section (I),
Toxicology Branch II/HED (H-7509C)
Signature: Stephen C. Dapson
Date: 9/24/92

EPA Section Head: Yiannakis M. Ioannou, Ph.D.
EPA Review Section (I),
Toxicology Branch II/HED (H-7509C)
Signature: Y M Ioannou
Date: 10/8/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

EPA Pesticide Chemical Code: 030703

Tox Chem. Number: 780A and 592

MRID Number: 400691-05

TEST MATERIAL: Sodium alanap

SYNONYM: Naptalam

SPONSOR: Uniroyal Crop Protection, Bethany, CT

STUDY NUMBER: 860015-10

TESTING FACILITY: American Biogenics Corp., Woburn, MA

TITLE OF REPORT: Micronucleus Assay With Sodium Alanap

AUTHOR: K.S. Loveday

REPORT ISSUED: January 28, 1987

CONCLUSIONS--EXECUTIVE SUMMARY: No definitive conclusions can be reached from the mouse micronucleus assay conducted with males and females administered a single oral gavage dose of 1500 mg/kg sodium alanap. The data suggested a time-related increase in micronucleated polychromatic erythrocytes (MPEs) with a maximum response occurring 2 days postexposure in the females and 3 days postexposure in the males. Without historical background frequencies, we are unable to determine whether the increases in MPEs fell within the normal range for the reporting laboratory or are indicative of a genotoxic response. It was noteworthy, however, that the increase in MPEs over time was consistent with the unequivocal evidence of sodium alanap-induced cell-cycle delay and clastogenesis in cultured Chinese hamster ovary (CHO) cells (see DER 91-03). The interpretation of the data was further confounded by the overall lack of agreement between the LD_{50}, the preliminary toxicity test, and the micronucleus assay survival data (see Section C--Reported Results and Section D--Reviewers' Discussion/Conclusions). The results, therefore, did not provide full assurance that doses were accurately prepared or that animals were treated with an appropriate concentration of the test substance.

Additionally, there was no information on the purity of the test material. Based on the above considerations, we conclude that the study is unacceptable.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category II, Structural Chromosomal Aberrations. It is recommended that the repeated assay be conducted with a high level that clearly demonstrates the maximum tolerated dose for both sexes. In light of the suggestive evidence of a time-related increase in MPEs, it is further recommended that three doses be assayed for the determination of a possible dose-dependent response. The study report should also include information on test material purity and all clinical signs including death.

A. MATERIALS:

1. Test Material: Sodium alanap

Description: Purple powder
Identification Number: Lot/Batch Number: DJS-050586
Purity: Not reported
Receipt date: May 8, 1986
Stability: Stable at room temperature (see DER 9-64)
Contaminants: None listed
Solvent used: Deionized water (DH_2O)
Other provided information: The test material was stored at room temperature. The frequency of dosing solution preparation was not reported.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: DH_2O was administered by oral gavage at a dosing volume of 5 mL/kg.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was prepared in DH_2O and administered by oral gavage at 60 mg/kg; dosing volume = 5 mL/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Preliminary toxicity test: 90, 225, 425, 855, and 1280 mg/kg
- Micronucleus assay: 500, 750, and 1500 mg/kg

4. Test Animals:

(a) Species: mouse Strain: CD-1
Age: 38 days (males); 46 days (female)--preliminary toxicity test;
49 days (males and females)--micronucleus assay
Weight range: (day prior to treatment): 21.1-31.1 g (males);
19.7-25.9 g (females)--preliminary toxicity test
(day prior to treatment): 26.4-34.6 g (males);
22.7-32.0 (females)--micronucleus assay
Source: Charles River Breeding Laboratories, Wilmington, MA

(b) Number of animals used per dose:

- Preliminary toxicity test: 5 males; 5 females per treatment group
- Micronucleus assay: 15 males; 15 females per treatment group
  10 males; 10 females (vehicle control group)
  5 males; 5 females (positive control group)

Note: Dosing was based on individual body weights; these data were not provided.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x (Day 1) x (Day 2) x (Day 3)

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ x Day 2

(c) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ x Day 2

2. Tissues and Cells Examined:

x bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 200

CC139

3. Details of Slide Preparation: At 1, 2, and 3 days after administration of the test material, the appropriate group of animals were sacrificed by cervical dislocation. Sacrifice time for the vehicle and positive control groups was day 2 posttreatment. Bone marrow cells were aspirated from both femurs mixed with fetal calf serum (FSC) and centrifuged. Supernatants were removed; cell pellets were resuspended and spread onto slides. Prepared slides were stained using either Stat Stain or Camco-Quick (methanol:Wright-Giemsa) and coded with the randomly assigned animal numbers.

4. Statistical Methods: The results were evaluated for statistical significance using the Cochran-Armitage test.

5. Evaluation Criteria: No criteria were provided to establish the validity of the assay or the biological significance of the findings.

6. Protocol: None provided

C. REPORTED RESULTS:

1. Preliminary Toxicity Assay: Dose selection for the preliminary range-finding study was based on information supplied by the sponsor indicating that the expected LD_{50} for CD-1 male and female mice was 620 and 1741 mg/kg, respectively. Additional information supplied by the sponsor stated that "Doses of 2500 and 5000 mg/kg were extremely lethal to the mice and some deaths were observed at 1200 mg/kg and 600 mg/kg." Accordingly, the oral gavage doses used in the preliminary assessment were 90, 255, 425, 855, and 1280 mg/mL sodium alanap. Following administration of the selected doses to groups of five male and five female mice, animals were observed daily for 5 days. One male in the high-dose group died on day 2; no other deaths occurred in the male mice. One high-dose female was prostate on day 5 and subsequently died after completion of this phase of testing (day 9). Other deaths occurring among the females included three in the 855-mg/kg group and one in the 255-mg/kg group. The examination of bone marrow cells from two males dosed with 855 mg/kg and two females receiving 425 mg/kg revealed no adverse effects on PCEs or NCEs. The study author stated that since the survival data from the range-finding test conflicted with the reported LD_{50} information, 500, 750, and 1500 mg/kg sodium alanap were selected for the micronucleus assay.

2. Micronucleus Assay: Two males administered the high-dose and one male administered the mid-dose died prior to the scheduled sacrifice; no deaths were observed in the treated females. Based on these findings, bone marrow cells harvest from males and females 1, 2, and 3 days postexposure to 1500 mg/kg of the test material were analyzed for micronuclei induction. As the data presented in Table 1 show, the percentage of PCEs in the treatment group at all harvest times for both sexes were comparable to the vehicle control values. However, a time-related increase in MPEs was seen in the males with the maximum

TABLE 1. Results of the Micronucleus Assay in Mice Treated with Sodium Alanap

Substance	Dose/kg	Exposure Time[a] (day)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group[b]	MPEs per 1000 PCEs	% PCEs per group[b]
Vehicle Control								
Deionized water	5 mL	2	M	10	10,000	18	1.8±1.5	59.9
		2	F	10	10,000	12	1.2±0.92	62.3
Positive Control								
Cyclophosphamide	60 mg	2	M	5	5000	64	12.8±4.3	42.0
		2	F	5	5000	94	18.8±15.2	31.3
Test Material								
Sodium alanap	1500 mg[c]	1	M	4	4000	4	1.0±1.41	58.6
		1	F	5	5000	8	1.6±1.67	58.1
		2	M	5	5000	10	2.0±1.87	64.1
		2	F	5	5000	17	3.4±1.14	59.9
		3	M	4	4000	11	2.75±1.71	52.1
		3	F	5	5000	5	1.0±0.71	60.7

[a]Time after compound administration by oral gavage
[b]Calculated by our reviewers
[c]Highest assayed dose; bone marrow cells were not analyzed from males and females in the mid- (750) mg/kg) or low- (500 mg/kg) dose groups. Two males in the high-dose group and one male in the mid-dose group died prior to the scheduled sacrifice.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes

yield of MPEs occurring at the final cell harvest. In females, the response peaked at day 2 and subsequently declined. The study author did not comment on the ~3-fold increase in MPEs recorded for the females sacrificed 2 days posttreatment. However, using the concurrent background frequency (1.2 MPEs/1000 PCEs) for females in conjunction with the criterion of Salamone et al.[1] for evaluating micronucleus assay data, which assumes a spontaneous frequency of 1.28 MPEs/1000 PCEs for mice, a group total of ≥14 MPEs (2.8 MPEs/1000 PCEs) for five animals would be considered suggestive of a positive response. Both the total number of MPEs (17) and the frequency of MPEs (3.4±1.14) for the females at the 2-day interval exceeded the corresponding values listed above. Similarly, the MPE frequency for males in the 72-hour harvest group (2.75±1.71) is suspect. We are, nevertheless, unable to reach a definitive conclusion because historical control data were not provided and spontaneous MPEs frequencies are known to vary widely among laboratories. In a collaborative study with 15 participating laboratories, the spontaneous frequencies for mice ranged from a low of 1.2 to a high of 4.9 MPEs per 1000 PCEs.[2]

The study author concluded, however, that "Sodium Alanap is not capable of inducing micronuclei in mouse bone marrow cells."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: As previously stated, no definitive conclusions relative to the genotoxic potential of sodium alanap can be reached from this study. Without historical control data, we are unable to determine whether the time-related increases in micronuclei induction observed in both sexes, compared to the concurrent controls, are within the normal variation of the performing laboratory or are indicative of a clastogenic effect. It was noteworthy that unequivocal evidence of cell-cycle suppression and clastogenesis was demonstrated in the in vitro Chinese hamster ovary (CHO) cell cytogenetic assay conducted by the same laboratory with the identical lot of the test substance (see DER 93-63). It would, therefore, appear that the increase in MPEs overtime is consistent with the dramatic increase in chromosome aberrations found in treated CHO cells following a delayed 17-hour cell harvest.

Similarly, our reviewers have concerns regarding the overall lack of concordance in the survival data. The LD_{50} information provided by the sponsor indicated that sodium alanap was more toxic to male mice (LD_{50}--620 mg/kg) than female mice (LD_{50} 1741 mg/kg). However, in the preliminary toxicity test of doses up to 1280 mg/kg, the trend was reversed; more deaths occurred in the females but mortality was not dose dependent (three females in the 855-mg/kg group died within 3 days of

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[1]Salamone, M.F., Heddle, J.A., Katz, M. (1980). Mutagenic activity of 41 compounds in the in vivo micronucleus assay. In: F.J. de Serres and J. Ashby, eds. Progress in Mutation Research, Vol. 1, Elsevier, North Holland, 686-697.

[2]MacGregor, J.T., Heddle, J.A., Hite, M., Margolin, P.H., Ramel, C., Salamone, M.F., Tice, R.R., Wild, D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutat. Res. 189:103-112.

exposure and one female in the 1280-mg/kg group died 9 days posttreatment). Among the five preliminary treatment-group males, only one animal at the high dose died. Although deaths in treated males occurred in the micronucleus assay (2 males at 1500 mg/kg and one male at 750 mg/kg), no signs of compound toxicity were reported for the females.

Based on the above considerations, we conclude that the inability to reproduce a sex-specific survival trend provides no assurance that the doses were accurately prepared or that the animals received the appropriate high dose. The study is unacceptable and should be repeated. In addition, there was no information on the purity of the test material.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated December 9, 1986).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category II, Structural Chromosomal Aberrations.

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Pages 134 through 139 are not included in this copy.

The material not included contains the following type of information:

__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

__ _ __ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

__ _ __ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

__ _ __ Internal deliberative information.

_____ Attorney-Client work product.

__ _ __ Claimed Confidential by submitter upon submission to the Agency.

DOC930162

FINAL

009803

DATA EVALUATION REPORT

NAPTALAM

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 9/23/92

Independent Reviewer Lynne T. Haber, Ph.D. Date 9/23/92

QA/QC Manager Sharon Segal, Ph.D. Date 9/23/92

00140

Contract Number: 68D10075
Work Assignment Number: 1-110
Clement Number: 93-66
Project Officer: James Scott

009303

GUIDELINE §84 MUTAGENICITY
UDS

EPA Reviewer: Stephen C. Dapson, Ph. D
EPA Review Section (I.
Toxicology Branch II HED (H-7509C)
Signature: Stephen C. Dapson
Date: 9/28/92

EPA Section Head: Yiannakis M. Ioannou, Ph D
EPA Review Section (I.
Toxicology Branch II HED (H-7509C)
Signature: Y M Ioannou
Date: 10/2/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION NUMBERS.:

EPA Pesticide Chemical Code: 030703

Tox Chem. Number: 780A and 592

MRID Number: 401498-01

TEST MATERIAL: Sodium alanap

SYNONYM: Naptalam

SPONSOR: Uniroyal Chemical Co., Inc., Bethany, CT

STUDY NUMBER: T5270.380

TESTING FACILITY: Microbiological Associates, Inc., Bethesda, MD

TITLE OF REPORT: Unscheduled DNA Synthesis assay in Rat Primary Hepatocytes Test Article Sodium Alanap Lot No. DJS-050586

AUTHOR: Curren, R.D.

REPORT ISSUED: March 31, 1987

CONCLUSIONS-EXECUTIVE SUMMARY: Concentrations ranging from 3 to 300 µg/mL sodium alanap did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. Doses ≥100 µg/mL were insoluble and levels ≥1000 µg/mL were severely cytotoxic. It was concluded, therefore, that sodium alanap was tested over an appropriate range of concentrations and failed to induce a genotoxic response. Although the assay was performed in a technically acceptable manner, the study is incomplete because information on the purity of the lot of sodium alanap used in this study was not provided.

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.4) for genetic effects, Category III, Other Mutagenic Mechanisms but can be upgraded if the missing test material purity information is provided.

C0141

A. MATERIALS:

1. Test Material: Sodium alanap

Description: Light purple powder
Identification number: Lot number: DJS-050586
Purity: Not reported
Receipt date: October 20, 1986
Stability: Stable at room temperature. (see Data Evaluation Record 93-64)
Contaminants: None listed
Solvent used: Williams Medium E (WME)
Other provided information: The test material was stored at room temperature and solutions used in the assay were prepared immediately prior to use.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of one adult male Fischer 344 rat (preliminary cytotoxicity assay) and one adult male Sprague-Dawley rat (UDS assay) obtained from the Charles River Laboratories and the Fredrick Cancer Research Facility, Frederick, MD, respectively.

3. Control Substances: WME was used as the solvent control for the test compound; dimethyl sulfoxide (DMSO) at 10 µL/mL was used as the solvent control for the positive control. 7,12-Dimethylbenzanthracene (DMBA) at 3 and 10 µg/mL was used as the positive control.

4. Medium: WME: Williams Medium E with 2 mM L-glutamine and antibiotics; WME+: WME with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: 0.3, 1, 3, 10, 31, 103, 308, 1025, 3075, and 10,250 µg/mL.

(b) UDS assay: 3, 10, 30, 100, 300, 1000, 3000, and 10,000 µg/mL; cells exposed to levels ≤300 µg/mL were scored for UDS.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: Rats were anesthetized with metofane. The livers were perfused with Hank's balanced salt solution containing 0.5 mM EGTA, pH 7.3 and with WME containing 80 units/mL collagenase and Hepes buffer (pH 7.3). Livers were excised, shaken in the collagenase perfusion solution, and either combed to release the hepatocytes or passed through a stainless-steel sieve.

(b) Hepatocyte harvest/culture preparation: Recovered cells were collected, counted and seeded in WME+ at a density of

≈$5x10^5$ cells, either into preconditioned 35-mm tissue culture dishes for the cytotoxicity assay, or onto coverslips in 35-mm tissue culture plates for the UDS assay. Cultures were placed in an incubator for 90-120 minutes, washed and fed WME prior to use.

2. Preliminary Cytotoxicity Assay: Duplicate hepatocyte cultures were exposed to ten doses of the test compound, ranging from 0.3 to 10,250 µg/mL or the solvent control (WME) for 18-20 hours. Following exposure, aliquots of the treatment medium were removed, centrifuged and measured for lactic acid dehydrogenase (LDH) activity. Relative cytotoxicity was assessed by subtracting the LDH activity of the solvent control from the LDH activity in the treated cultures and comparing the values to the amount of LDH released by exposure of high-dose cultures or the solvent control cells to 1% Triton.

3. UDS Assay:

   (a) Treatment/Slide Preparation: Five prepared hepatocyte cultures (2 cultures seeded into culture dishes and 3 cultures seeded onto coverslips) were exposed for 18-20 hours to eight selected doses (3-10,000 µg/mL) of the test material, the solvent control for the test material (WME), the solvent control for the positive control (DMSO), or the positive control (DMBA). Treatment medium contained 10 µCi/mL [^{3}H]thymidine. Monolayers grown directly on dishes were used to assess LDH activity as described for the cytotoxicity assay. Treated hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed in ethanol-glacial acetic acid, dried and mounted.

   (b) Preparation of Autoradiographs/Grain Development: Slides were dipped into Kodak NTB emulsion, dried and stored at 4°C in slide boxes containing a desiccant for 11 days. Slides were developed in Kodak D-19, fixed, stained with hematoxylin-sodium acetate-eosin, coded and counted.

   (c) Grain Counting: The nuclear grains of 75 randomly selected cells with appropriate background counts and normal morphology (25/slide) from each test, negative, and positive control group were scored for the incorporation of tritiated thymidine into DNA. Net nuclear grain counts were determined by subtracting the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count of each cell. Means and standard deviations were calculated for each treatment group.

4. Evaluation Criteria:

   (a) Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the number of cells in repair in the negative control must be <15% and (2) the positive control compound must induce a significant increase in the net nuclear grain count (≥5 grains/nucleus over the negative control).

(b) Positive Response: The assay was considered positive if the test material induced a dose-related increase in mean net nuclear grains and one or more of the doses had an increase in the mean net nuclear grain count that was ≥5 grains/nucleus over the negative control. In the absence of a dose-related effect, a compound that showed nuclear grain counts that were ≥5 grain/nucleus over two successive doses was also considered positive.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Ten doses (0.3-10,250 µg/mL) of the test material were examined in the cytotoxicity assay. The study author stated that compound precipitation was observed at the five highest concentrations (103, 308, 1025, 3075, and 10,250 µg/mL). As shown in Table 1, cytotoxicity, as indicated by increased leakage of LDH into the culture medium, did not proceed in a conventional dose-related manner. At the highest assayed concentration, no cytotoxicity was seen; however, as the dose was reduced, LDH values increased slightly at 3075 and 1025 µg/mL with a subsequent decline at ≤308 µg/mL. These results, which conflicted with the microscopic evidence of cytotoxic effects on the monolayer at all assayed levels, suggest that the physical state of the test material interfered with the LDH determinations. Additionally, the low relative cytotoxicity (16%) for high-dose cultures treated with 1% Triton supports this assessment. Based on these findings, the study author selected a 3-10,000-µg/mL dose range for the UDS assay.

2 UDS Assay: In agreement with the preliminary results, doses ≥100 µg/mL were insoluble and the LDH values did not correspond to the microscopic evidence of severe cytotoxicity at the three highest concentrations (1000, 3000, and 10,000 µg/mL). These levels were not scored for UDS. Results from the analysis of hepatocytes exposed to sodium alanap concentrations ranging from 3 to 300 µg/mL showed no appreciable increase in net nuclear grains or the percentage of cells with ≥5 net nuclear grains. By contrast, the selected doses of the positive control (3 and 10 µg/mL) induced marked increases in UDS. The study author concluded, therefore, that sodium alanap was negative in the primary rat hepatocyte UDS assay.

Representative results are presented in Table 2.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was well-conducted and the author correctly interpreted the data. Sodium alanap was assayed over a concentration range that included insoluble levels (≥100 µg/mL) and severely cytotoxic levels (≥1000 µg/mL) but failed to induce a genotoxic response in primary rat hepatocytes. The failure to demonstrate compound cytotoxicity by the analysis of LDH activity did not compromise the study, since there was sufficient evidence from the gross

TABLE 1. Representative Results of the Preliminary Cytotoxicity Assay with Sodium Alanap: Lactate Dehydrogenase (LDH) Activity

Treatment	Dose (µg/mL)	Average LDH Activity (units/L)[a]	Corrected LDH Activity (units/L)[b]	Relative Percent Cytotoxicity[c]
Solvent Control				
Culture medium	--	190.5	0	0
Culture medium +1% Triton	--	969.5	779.0	100
Test Material Control				
Sodium alanap +1% Triton	10,250	311.5	121.0	16
Test Material				
Sodium alanap	31[d]	236.5	46.0	6
	103	310.5	120.0	15
	308	333.5	143.0	18
	1025	386.5	196.0	25
	3075	261.5	71.0	9
	10,250	163.5	-27.0	-3

[a]Average of two samples

[b]Corrected LDH = Average LDH of Test Groups - Solvent Control LDH

[c]Relative Percent Cytotoxicity = $\frac{\text{Corrected LDH of Test Group}}{\text{Corrected LDH of Solvent Control +1\% Triton}} \times 100.$

[d]Highest soluble concentration; LDH values for lower levels (0.3, 1, 3, and 10 µg/mL) did not indicate cytotoxicity. Adverse effects were, however, reported on monolayers exposed to all assayed concentrations.

TABLE 2. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with Sodium Alanap

Treatment	Dose/mL	Cytotoxicity			UDS Activity		
		Average LDH Activity (units/L)[a]	Corrected LDH Activity (units/L)[b]	Relative Percent Cytotoxicity[c]	Number of cells Scored[d]	Mean Net Nuclear Grain Count ± S.D.	Percent cells with ≥5 Grains
Solvent Control (Test Material)							
Culture medium	--	82.5	0.0	0	75	0.1±2.2	3
Culture medium +1% Triton	--	385.0	302.0	100	--	--	--
Solvent Controls (Positive Control)							
Dimethyl sulfoxide	10 µL	93.5	0.0	0	75	-0.6±3.5	4
Positive Control[e]							
7,12-Dimethylbenzanthracene	3 µg/mL	99.0	5.5	2	75	16.2±6.2	100
Test Material							
Sodium Alanap	30 µg[f]	175.0	92.5	31	75	0.9±2.4	4
	100 µg[g]	175.5	93.0	31	75	1.2±2.3	7
	300 µg[h]	133.5	51.0	17	75	0.7±1.4	0

[a]Average of duplicate samples

[b]Corrected LDH = Average LDH of Test Group - Solvent Control LDH.

[c]Relative Percent Cytotoxicity = $\frac{\text{Corrected LDH of Test Group}}{\text{Corrected LDH of Solvent Control +1\% Triton}} \times 100$.

[d]Twenty-five cells were scored per culture (3 cultures/group).

[e]Comparable results were obtained with 10 µg/mL of the positive control; the data from the lower dose were, therefore, selected as representative.

[f]Findings for lower doses (3 and 10 µg/mL) did not suggest a genotoxic effect.

[g]Highest soluble concentration

[h]Higher levels (1000, 3000, and 10,000 µg/mL) were not scored because of severe cytotoxic effects on the monolayers.

examination of the hepatocytes to indicate that the doses evaluated in the UDS assay were correctly chosen. In addition, the sensitivity of the test system to detect UDS was adequately demonstrated by the findings with the positive control (3 and 10 µg/mL DMBA). Although we concluded that sodium alanap was evaluated in an acceptable assay and found to be not genotoxic, the study is incomplete because information on the purity of the test material was missing.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated March 25, 1987).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-9; Appendix B, Protocol, CBI pp. 18-24.

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms but can be upgraded if the missing test material purity information is submitted.

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