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Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 11/16/93
Senior Pharmacologist, Review Section I, TBII/HED H7509C

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. 11/17/93
Section Head, Review Section I, TBII/HED H7509C

DATA EVALUATION RECORD

Study Type: Acute Dermal Toxicity; Species: Rabbit; Guideline: §81-2

EPA Identification No.s:
EPA MRID# 427397-01
EPA Pesticide Chemical Code 030703 2
Toxicology Chemical Code 780A
EPA DP Barcode D192054
EPA Submission No. S442249

Test Material: Alanap Technical (Lot# DJS/1/65A)

Synonyms: Naptalam

Sponsor: UNIROYAL CHEMICAL COMPANY, INC., 74 Amity Road, Bethany, CT 06524-3402

Testing Facility: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

Title of Report: ACUTE DERMAL TOXICITY IN RABBITS/LD_{50} IN RABBITS

Study Number(s): MB 93-2326 B

Author(s): D.R. Cerven

Report Issued: April 1, 1993

Conclusions: In an acute toxicity study, New Zealand White Rabbits were dermally exposed to 5000 mg/kg Alanap Technical (≈52% a.i.) for 24 hours and then observed for a period of 14 days. **The dermal LD_{50} in rabbits for Alanap Technical is greater than 5000 mg/kg.**

The study is classified as **Core Minimum Data** with a **TOXICITY CATEGORY IV** and **satisfies** the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 81-2) for a dermal toxicity (LD_{50}) study in rabbits.

A. Materials and Methods A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:
Purity: ≈52 % a.i. (see below)
Density: not provided
Description: purple powder
Lot No.: DJS/1/65A
Receipt date: 1/25/93
Other provided information: stored at room temperature and humidity
Contaminants: no data provided

The registrant stated in a fax sent 8/25/93 that:

[redacted] The dried product was assayed for Naptalam and was found to contain 90.48% active ingredient. The report of the analysis of the dried product was submitted under MRID No. 418388-01.

Vehicle(s): distilled water

Test Animal(s):
Species: Rabbit
Strain: New Zealand White
Source: Ace Animals
Age: not provided
Body Weight: 2.3-2.9 kg-females; 2.4-2.7 g-males

B. Study Design

This study was designed to assess the acute dermal toxicity (LD_{50}) in rabbits.

Animal Husbandry

Animals were acclimated to the laboratory conditions for approximately 1 week and singly caged under standard animal care conditions. The animals received Purina Rabbit Chow (Diet #5321) and water *ad libitum*.

Dose Administration:

Five males and 5 females received a dose of 5000 mg/kg. *Approximately 24 hours prior to application of the test article, the dorsal area of the trunk of each animal was clipped free of hair. The prepared site was approximately 10% of the body surface and remained intact. The test article was moistened...applied to the prepared dermal site...covered with a gauze patch, secured with non-irritating tape...*following which the...*torso was wrapped with plastic which was secured with non-irritating tape. After 24 hours, the patches were removed. Any residual test article was removed by gentle washing with distilled water...*

Observations

According to the investigators: *The test sites were scored for dermal irritation at 24 hours post dose and on days 7 and 14 using the numerical Draize scale. Additional signs were described. The animals were observed 1, 2 and 4 hours post dose and once daily for toxicity and pharmacological effects. The animals were observed twice daily for 14 days for mortality. Body weights were recorded pretest, weekly and at death or termination. All animals were examined for gross pathology. Abnormal tissues were preserved in 10% buffered formalin for possible future histopathic examinations.*

Statistical analysis

No statistical analysis methods were employed.

Compliance

A signed and dated Statement of No Data Confidentiality Claims was provided.

A signed and dated Good Laboratory Practice Statement was provided.

A signed and dated Quality Assurance Evaluation Statement was provided.

A signed and dated FIFRA Flagging Statement **was not** provided; however, it is the opinion of this reviewer that this study neither meets not exceeds any of the applicable criteria.

C. Results

1. Mortality

No mortality was reported.

2. Body Weights

Group summary and individual animal data were provided by the investigators, no treatment related effects were noted.

3. Dermal Reactions

The following table from the investigators report presents the dermal reaction data:

Eartag # & Sex	1 R	1 E	7 R	7 E	14 R	14 E	% Rem.
D5974/M	0a	0	0a	0	0a	0	100
D6076/M	3ab	2	0*	0	0*	0	100
D6082/M	1ab	2	0a	0	0a	0	100
D6086/M	3ab	2	0*	0	0*	0	100
D6085/M	2ab	2	1	0	1*	0	100
D6003/F	2a	2	1a	0	0a	0	100
D6127/F	2ab	1	0	0	0	0	100
D6126/F	2ab	2	1a	0	1a	0	100
D6125/F	2ab	2	2a	0	1a	0	100
D6129/F	2ab	2	0	0	0*	0	100

CODE: R = erythema (redness)
E = edema
a = dose site stained purple
b = small amount of test article remaining on dose site
* = animal reclipped
% Remaining = a visual estimate of the amount of material remaining on the skin, gauze and occlusive binding at 24 hours, after the occlusive binding was removed.

4. Clinical Signs of Toxicity

No treatment related effects were noted.

5. Necropsy Observations

Two females and 1 male had treatment related skin abnormalities.

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D. **Discussion/Conclusions**

The dermal LD_{50} in rabbits for Alanap Technical (≈52% a.i.) is greater than 5000 mg/kg.

E. **Study Deficiencies**:

No specific deficiencies.

F. **Core Classification**: **Core Minimum Data**.

TOXICITY CATEGORY IV

This study satisfies the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 81-2) for a dermal toxicity (LD_{50}) study in rabbits.

Page _____ is not included in this copy.

Pages __6__ through __8__ 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.

Guideline Series 84-2(a): **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 11/10/93
Section II, Toxicology Branch II (H7509C)
Secondary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 11/10/93
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT I

CHEMICAL: Sodium alanap Tox. Chem. No.: 780A

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

MRID NUMBER: 400691-03 (original report); 427397-03 (response to previous review)

SYNONYMS/CAS No.: Naptalan

SPONSOR: Uniroyal Chemical Company

TESTING FACILITY: American Biogenics Corporation

TITLE OF REPORT: CHO/HGPRT Mutagenesis Study with Sodium Alanap

AUTHOR: Story, D. L.

STUDY NUMBER: 8600 15-30

REPORT (RESPONSE) ISSUED: April, 1993

BACKGROUND: The original report (in MRID 400691-03) was reviewed (Caswell review no. 009801) and signed off in October, 1992. The DER noted that: "The study was...seriously compromised because of the marked differences in the cytotoxicity data obtained from the preliminary and mutational assays... our reviewers assume that the inability to reproduce reasonably comparable cytotoxicity results probably resulted from substandard culture conditions rather than test material instability... In addition, the purity of the test material was not provided."

CLASSIFICATION: Upgraded to Acceptable. The information provided in MRID 427397-03, particularly the discussion relating to the toxicity curves associated with exposure of the CHO cells to the test material, adequately explains the marked differences in the cytotoxicity observed between the preliminary and mutagenicity assays. In addition, information has been submitted relating to the purity of the test material (sodium alanap, 91.8%, detectable impurities, [redacted] Despite the relatively poor cloning efficiency for the background control cultures (≈50% -S9, 44.7% +S9

in the preliminary cytotoxicity test; 55.5% -S9, 43.4% +S9 in the mutation assay), the considerably reduced % of cells capable of replication (4% and 11%) following exposure to 1500 µg/ml, indicates that the test material was assayed to a reasonably adequate cytotoxic level. There was no indication of a mutagenic response in the CHO cells at the HGPRT locus. This study now satisfies the Guideline requirements (§84.2) for genetic effects, category I, gene mutations.

CONCLUSION(S):

1. The test material is adequately characterized.

2. Sufficient information has been received regarding the variability in cytotoxicity (with findings from the terminated mutagenesis assay consistent with those from the subsequent reported assay). While the cloning efficiency (approximately 50%) was somewhat lower than desirable, there is no indication that this reduction could have compromised the study.

A. MATERIALS

1. Test Material: Designated as DJS-050586, consisting of 91.8% sodium alanap, [redacted] The test material is described in the previous review as a "purple powder."

B. DISCUSSION

Most of the relevant material in MRID 427397-03 consists of a letter (dated February 19, 1993) and relevant tables from Dr. Kenneth S. Loveday (the author of the original report in MRID 400691-03). Regarding the original review comment: "The study was seriously compromised because of the marked differences in the cytotoxicity data obtained from the preliminary and mutational assays" the following response is given:

"We agree that there were some differences in the cytotoxicity results between the range-finding studies and the mutation studies using nonactivated conditions, but there was general agreement in the shape of the cytotoxicity curve. The differences, however, were not due to substandard culture conditions in the mutagenesis assays, but reflect variability based on the nature of the test chemical. This variability may have been exaggerated since the range-finding cytotoxicity studies used single flasks while the mutagenicity studies used duplicate flasks. An additional nonactivated mutagenicity assay was conducted (but not reported due to cytotoxicity) and the cytotoxicity results of that assay were consistent with the results from the mutagenicity study presented in the final report."

While it is not immediately evident why use of single flasks would result in reduced (although it could result in a greater variability in) cytotoxicity as compared to experiments using duplicate flasks, there was 26% cell recovery at 1990 µg/ml in the first (terminated) mutagenesis study with sodium alanap, although cells could not be recovered from this culture 3 days later for replating during the expression period. In the subsequent (reported) mutagenesis assay 51% of the cells were recovered at 1500 µg/ml, and the percentages of survivors from the recovered cells were 4 and 11%. Refer to appended pages 1 and 2 for the summaries of toxicity data from the terminated and subsequently reported mutagenesis assays.

The discussion also states that the cloning efficiency of CHO cells (which was, although low, reasonably consistent) is dependent on the specific lot of fetal calf serum. In addition, the statement is made that the variability in cytotoxicity may reflect the toxicity curve(s) generated from sodium alanap ("a broad shoulder with a steep slope"), and "with chemicals of this type, it is often difficult to select the concentrations which will reproducibly kill 50% or 90% of the cells. The general shape of the toxicity curves, however, are reproducible from experiment to experiment."

Overall then, sufficient information has been received regarding the variability in cytotoxicity (with findings from the terminated mutagenesis assay consistent with those from the subsequent reported assay). While the cloning efficiency (approximately 50%) was somewhat lower than desirable, there is no indication that this reduction could have compromised the study. The study classification is upgraded to acceptable. This study now satisfies the Guideline requirements (§84.2) for genetic effects, category I, gene mutations.

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Table 2: Summary of Toxicity Data from Terminated Mutagenesis Study with Sodium Alanap (nonactivated)

Concentration (µg/ml)	Recovered Cells (x 10^4)	% Recovery
4980	0.5/0.5	0.2%
4480	ND/5.0	2.0%
3980	ND/10.0	4.0%
2990	1.5/1.0	0.6%
1990	95.0/20.2	26% *
995	100/85	41%
497	160/370	118%
0	300/140/280/180	100%

ND, not determined; accurate cell count could not be made using hemacytometer due to cellular debris

Average of vehicle control; 225 x 10^6 cells recovered/flask, defined as 100% recovery

*, three days later, no cells were recovered for replating during expression period

Table lists number of recovered cells following 16-hour exposure to test chemical and to vehicle control. 1.3 x 10^6 cells were seeded on Day 1; cells were exposed to chemical on Day 2, and total number of recovered cells was determined on Day 3, following removal of test chemical. Number of recovered cells does not represent number of survivors since not all recovered cells would form colonies during clonal assay.

Clonal survivors were not determined from number of recovered cells since experiment was terminated. Experiment was terminated due to toxicity of Sodium Alanap at concentrations of 1990 µg/ml and higher.

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Table 3: **Summary of toxicity data from reported mutagenesis assay with sodium alanap (nonactivated)**

Conc (ug/ml)	Recovered Cells x 10^4	% Recovered	% Survivors from Recovered cells (clonal data)
1500	340/590	51%	4% and 11%
1000	560/590	63%	51% and 52%
500	850/108	106%	108% and 99%
300	1290/1120	132%	83% and 74%
150	1320/1150	134%	75% and 88%
100	830/1150	109%	87% and 90%
50	1030/850	103%	53% and 88%
30	1340/1130	136%	77% and 107%
15	1260/1070	128%	112% and 93%
0	1080/850/ 810/910	100%	100%

Vehicle control average was 9.1 x 10^6 cells/flask and defined as 100% recovery

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Chemical:	**Benzoic acid, 2-((1-naphthalenylamino)ca**
PC Code:	**030702**
HED File Code	**13000 Tox Reviews**
Memo Date:	**11/17/94 12:00:00 AM**
File ID:	**DPD192054**
Accession Number:	**412-04-0046**

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