OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

TXR No.: 0051958

DATE: January 29, 2004

SUBJECT: **1-Naphthyl Acetamide: Review of Toxicity Studies**
PC Code 056001
Reregistration Case #: 0379

FROM: Abdallah Khasawinah, Ph.D., Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

THRU: Susan V. Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

TO: Mark Howards, Chemical Review Manager
Reregistration Branch 3
Special Review and Reregistration Division (7508C)

TASK ID: DP Code D213980, D214872, D210844, D223223, D228051, D293238

Action Requested: Review and Update of Toxicology Studies to Support Reassessment Eligibility Decision (RED)

Agency's Action:

HED has prepared/or updated the executive summaries of the Data Evaluation Records (DER's) on the subject studies in light of the new guidelines and classification systems. The updated executive summaries and DERs are attached. The study findings are listed below.

RC
03/04

1. Acute Oral Toxicity - Rat

In an acute oral toxicity study (MRID 00108833), groups of Sprague Dawley rats (5/sex) were given 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) in 0.25% methylcellulose by single dose gavage at the following doses of 1000, 1500, 2000, 2500 or 3000 mg/kg bw. Dosage volume was 5 ml/rat for the two lowest dosages and 10 ml/rat for the three highest dosage levels.

The oral LD_{50} (95% C.I.) for males and females combined= 1690 mg/kg (1408-2028 mg/kg) 1-Naphthyl Acetamide is classified as **TOXICITY CATEGORY III.** This acute oral toxicity study in the rat was originally classified core-minimum (HED 005378) but a later review in 1993 (HED 010667) considered it supplementary due to lack of purity description and the use of excessive dosing volumes exceeding the guideline requirements (1 ml/100 g bw). However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. The use of the excessive volume in dosing would exacerbate the toxicity of the test material. Because of this later deficiency, the study is classified **Unacceptable/Guideline.** A more recent **Acceptable/Guideline** study (MRID 43495901) satisfies this requirement.

2. Acute Oral toxicity Study -Rats

In an acute oral toxicity study (MRID 43495901), groups of fasted, young adult Sprague-Dawley rats (5/sex) were given a single oral dose of technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) at doses of 2000 or 5050 mg/kg bw by gavage. The test material was dosed as a 50% w/v mixture in 2% carboxymethyl cellulose (CMC) in deionized water and the animals were observed for 14 days.

Oral LD_{50}	Males > 5050 mg/kg bw
	Females > 5050 mg/kg bw
	Combined > 5050 mg/kg bw

Technical 1-Naphthaleneacetamide is in EPA **Oral Toxicity Category IV.** This acute oral toxicity study is classified as **Acceptable/Guideline.**

3. Acute Dermal Toxicity - Rabbit

In an acute dermal toxicity study (MRID 00108832), five male and five female New Zealand White rabbits received 2 g/kg of 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) on the abraded skin under occlusive wrap for 24 hour exposure.

The dermal LD_{50} in the rabbit in this test is greater than 2 g/kg. 1-Naphthyl Acetamide is classified as **CATEGORY III for dermal toxicity.** This acute dermal toxicity study in the rabbit was originally classified core-minimum (HED 005378) but a later review in 1993 (HED 010667) considered it supplementary due to lack or reported compound purity in the study report

and the use of abraded skin. However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. Although the use of abraded skin is not acceptable according to current standards of testing, the test material was moderately toxic under this extreme condition. Therefore the study is classified **Acceptable/Guideline**.

4. Acute Dermal Toxicity - Rabbit

In an acute dermal toxicity study (MRID 43495902), five male and five female young adult New Zealand White rabbits were dermally exposed to a dose of 2020 mg/kg bw technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) moistened with saline. The treated area was wrapped with surgical gauze secured with non-irritating adhesive tape for 24 hours.

Dermal LD_{50} **Males > 2020 mg/kg bw**
Females > 2020 mg/kg bw
Combined > 2020 mg/kg bw

Technical 1-Naphthaleneacetamide is in EPA **Dermal Toxicity Category III**. This acute dermal study is classified as **Acceptable/Guideline**.

5. Acute Inhalation Toxicity Study - Rat

In an acute inhalation toxicity study (MRID 43495903), groups of young adult HSD:SD rats (5/sex/group) were exposed by whole body inhalation to 1-Naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) for 4 hours at analytical concentrations of 0.710 or 2.17 mg/L. The animals were then observed for 14 days.

Inhalation LC_{50} **Males > 2.17 mg/L**
Females > 2.17 mg/L
Combined > 2.17 mg/L

Technical 1-Naphthaleneacetamide is classified in EPA **Inhalation Toxicity Category IV.** This acute inhalation study is classified as **Acceptable/Guideline**.

6. Acute Eye Irritation Study - Rabbit

In a primary eye irritation study (MRID 00103051), a dose of 100 mg of 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) was instilled into the lower right eyelid of nine New Zealand albino rabbits. The eyes of three rabbits were washed immediately after treatment. The other six eyes were unwashed.

NAA Acetamide is **corrosive** for the eyes and is classified as **Category I** for eye irritation (HED 005378). However a later HED review in 1993 (HED 010667) did not assign a toxicity category

because of a study deficiency in that the observation period did not continue for 21 days as the guidelines require. For this reason and the lack of information on the purity of the test material and the lot used, the reviewers considered this study **Unacceptable**. However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. Therfore, the present reviewer, believes that in spite of these deficiencies, the study demonstrates the corrosiveness of the test material and the study is **acceptable/guideline**.

7. Acute Eye Irritation - Rabbit

In a primary eye irritation study (MRID 43495904), 0.1 mL by volume (29 mg) of technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) was instilled into the conjunctival sac of the right eye of three male and three female young adult New Zealand White rabbits. The untreated eye served as a control. The animals then were observed for 72 hours.

Technical 1-Naphthaleneacetamide was minimally irritating to the eye based on the highest maximum mean irritation total score (4.3) recorded one hour after test material instillation. Therefore, technical 1-Naphthaleneacetamide **is classified in EPA Toxicity Category IV** for eye irritation. This study is classified as **Acceptable/Guideline**.

8. Skin Sensitization - guinea pigs

In a dermal sensitization study (MRID 43495905) with 5% v/v technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) in cottonseed oil, 15 male and 15 female Hartley albino guinea pigs were tested using the Magnusson and Kligman test.

After the intradermal and topical inductions, no dermal reactions were noted from any animal following challenge. In this study, Technical 1-Naphthaleneacetamide was not a dermal sensitizer. The study was conducted in a manner suitable to detect the sensitization potential of the test material. The results of a positive control study performed within six months of the current study were not reported. In spite of this deficiency, this study is classified as **Acceptable /Guideline**.

9. 90-Day Oral Toxicity Study - Rats

In a subchronic toxicity study (MRID 43896001), 1-Naphthaleneacetamide (Lot # I940415; 98.7% a.i.) was administered to CRL:CD BR rats (10/sex/dose) by feeding at dose levels of 0, 250, 1,000, or 4,000 ppm (mean measured concentrations of 0, 19.1, 73.8, or 292.1 mg/kg/day for males and 0, 20.4, 81.5, or 313.5 mg/kg/day for females) for 90 days.

In the 4,000 ppm treatment groups, mean body weights were lower for males (10-15%) and

females (9-12%) throughout the study, compared to controls. Final mean body weight gains were lower for males (14%) and females (20%). In addition, food consumption was consistently reduced for males (11-28%) and females (2-20%) throughout the study. Mean relative liver weights were significantly increased in both 4,000 ppm males (14%; $p \leq 0.05$) and females (32%; $p \leq 0.01$) with accompanying histopathological changes consisting of enlarged (hypertrophied) centrilobular hepatocytes with an abundance of fine granular eosinophilic cytoplasm. No rats died during the study. No treatment-related differences in clinical appearance, ophthalmology, hematology, clinical blood chemistry or urinalysis parameter or gross pathology were observed in any treatment group. No neoplastic tissue was observed in any of the treatment groups. The **LOAEL** is 4,000 ppm (292.1 mg/kg/day), based on decreased body weight, reduced body weight gain, reduced food consumption, and increased relative liver weights with histopathological changes in both sexes. The **NOAEL** is 1,000 ppm (73.8 mg/kg/day). This 90-day oral toxicity study in the rat is **Acceptable/Guideline**.

10. 90-Day Oral Toxicity Study - Dogs

In a subchronic toxicity study (MRID 43895901), 1-NAD (Lot/Batch # I940415; 98.7% a.i.) was administered via capsule to four beagle dogs/sex/dose at dose levels of 0, 30, 100, or 300 mg/kg/day for 13 weeks.

In the 300 mg/kg/day treatment group, all livers contained accumulations of a hemosiderin-containing pigment in the reticuloendothelial cells and bilirubin in the intracanicular spaces. The spleens of 3/4 males and 2/4 females also contained hemosiderin and hematopoiesis was increased in the bone marrow in 3/4 animals of both sexes. Decreases in red blood cell counts, hematocrit, and hemoglobin occurred in both sexes. Platelet counts and mean corpuscular volumes were increased in both sexes. Total bilirubin was increased in 1/4 males and 3/4 females, but the increases were significant ($p<0.05$ or 0.01) only for females. Body weights were reduced in males only. Clinical signs of toxicity in both sexes were soft or liquid feces. No treatment-related effects were observed in the 30 or 100 mg/kg/day treatment groups.

No dogs died during the study. No treatment-related differences in clinical appearance, food consumption, ophthalmology, urinalysis parameters, organ weights, or gross pathology were observed in any treatment group. No neoplastic tissue was observed in any of the treatment groups. The **LOAEL** is 300 mg/kg/day, based on increased platelet count, decreased red cell parameters, and increased mean corpuscular volume which correlate with histopathological changes observed in the liver, spleen, and bone marrow in both sexes. The **NOAEL** is 100 mg/kg/day. This 90-day oral toxicity study in the dog is **Acceptable/Guideline**

11. 21-Day Dermal Toxicity Study - Rat

In a repeated dose dermal toxicity study (MRID 43581001), 1-Naphthaleneacetamide (Lot # I940415; 98.71% ai) was applied to the shaved skin of Crl:CD BR rats (5/sex/dose) at dose levels of 0, 100, 300, or 1000 mg/kg for 6-6.5 hours/day, 5 days/week, for 3 weeks. No treatment-

related effects were observed at any dose level. There were no clinical signs of toxicity, and body weights, body weight gains, and food consumption were similar between the treated and control groups. No differences were observed in hematology parameters or clinical blood chemistry. Skin irritations occurred at similar rates in rats in all groups. Although males in the 1000 mg/kg treatment group had absolute liver weight which was 16.4% heavier ($p < 0.05$) than the control, no accompanying anatomical or functional changes were observed, and the mean liver weights of females in the 1000 mg/kg treatment group were lower than the control. No other differences in the organ weights, and no differences in macroscopic or microscopic organ morphology were observed between rats in the treated and the control groups. No neoplastic tissue was observed at any dose level. Ophthalmoscopic examinations and urinalysis were not performed during the study. No **LOAEL** was established. The **NOAEL** was the highest treatment level, 1000 mg/kg body weight. This 21-day dermal toxicity study in the rat is **Acceptable/Guideline.**

12. Bacterial Gene Mutation - *Salmonella typhimurium*

In a microbial/mammalian microsome plate incorporation mutagenicity study (MRID No: 43581006), *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to five doses of 1-naphthalene- acetamide technical (Lot # I 940415; 98.7%) ranging from 100 to 5000 µg/plate with and without S9 activation. Two independent trials were conducted. The S9 homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The test material was delivered to the test system in dimethyl sulfoxide.

Compound insolubility was seen at ≥3333 µg/plate +/-S9. Cytotoxicity was observed for all strains at 5000 µg/plate +/-S9 and for the majority of strains at 3333 µg/plate -S9. All strains responded in the expected manner to the appropriate positive control. There was, however, no indication that 1-naphthaleneacetamide technical induced a mutagenic effect at any dose with or without S9 activation. This study is classified as **Acceptable/Guideline.**

13. *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice

In a mouse micronucleus assay (MRID No: 43581005), groups of five male and five female ICR mice received single intraperitoneal injections of 250, 500 or 1000 mg/kg 1-naphthaleneacetamide technical (Lot # I940415; 98.7%). The test material was delivered to the animals as suspensions prepared in 1% aqueous carboxymethylcellulose. Animals were sacrificed at 24, 48 or 72 hours postexposure and bone marrow cells were harvested and examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

Overt toxicity in high-dose animals included death and lethargy. Slightly depressed polychromatic to normochromatic erythrocyte ratios (PCE:NCE) were also observed in both sexes of the high-dose group. The positive control induced the expected high yield of MPEs in males and females. There was, however, no indication that 1-naphthaleneacetamide technical induced a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. This study is

classified as **Acceptable/Guideline.**

14. *In Vitro* Mammalian Cell Gene Mutation Assay in L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells

In an *in vitro* mammalian cell forward gene mutation study (MRID No: 43580202), cultured L5178Y mouse lymphoma cells were exposed to doses of 1-naphthaleneacetamide technical (Lot # I940415; 98.7%) ranging from 10-2000 µg/mL +/-S9 (initial trial) and nonactivated doses of 100-2000 µg/mL or S9-activated levels of 10-250 µg/mL (confirmatory trial). The S9 homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity (i.e, ≤12% total viability) was seen at ≥1250 µg/mL -S9 and ≥150 µg/mL +S9. The positive controls induced the expected response in the target cells in both trials. There was no evidence of a mutagenic effect in the absence of exogenous metabolic activation. However, dose-related increases in the mutation frequency (MF) were seen at 100 and 250 µg/mL +S9 (MFs of 183 and $292x10^{-6}$ vs. the solvent control MF of $42x10^{-6}$ -- initial trial). These treatment group values represent MFs that were increased ≈4.4 and 7.0 fold, respectively. At the high dose, however, total growth was only 3%. In the S9-activated confirmatory trial, MFs of 63, 111, 107, 137 and $184x10^{-6}$ were calculated at 25, 50, 75, 100 and 150 µg/mL, respectively, vs. a background MF of $33x10^{-6}$. Fold increases ranged from 1.9 at 25 µg/mL (total growth =83%) to 5.6 at 150 µg/mL (total growth = 9%). These findings provide convincing evidence of mutagenesis. It is, however, not clear if the test material is also a clastogen. The induction of small colony mutants, which is thought to represent genetic damage not only at the TK locus but also at multiple linked loci on chromosome 11b, was only seen in the initial trial. We note that 1-naphthaleneacetamide technical was negative in the mouse micronucleus assay (see MRID No. 43581005). These findings are in agreement with the earlier results of a positive mouse lymphoma assay with the ethyl ester of 1-naphthaleneacetic acid (MRID 43580201). However, the issue as to whether the test substance has intrinsic clastogenic activity can only be resolved by performance of an *in vitro* cytogenetic assay. This study is classified as **Acceptable/Guideline.**

15. Metabolism and Pharmacokinetics - Rat

In a study (MRID 43963301) conducted to examine the metabolism and disposition of 1-naphthaleneacetamide, five male and five female Sprague-Dawley rats were given either a single 1 or 100 mg/kg bw oral dose, or a 14-day repeated dose (1 mg/kg/day). Groups of male and female rats were subjected to the dosing regimens above using [^{14}C] ring labeled -1-naphthaleneacetamide (Batch No. 94-516-38-10; 99.7% radiochemical purity, specific activity 55.5 mCi/mmol), and nonlabeled test article (Batch No. KP 0100487, chemical purity not available). Excretion, tissue distribution, and metabolite profiles were determined.

Overall recovery of administered radioactivity was an excellent 97.2-101%. 1-Naphthaleneacetamide was readily absorbed and excreted within 36 hours following a single 1

mg/kg bw, a 14-day repeat oral dose of 1 mg/kg bw, or a single 100 mg/kg bw oral dose. Following single or multiple oral low doses (1 mg/kg bw) of [C^{14}]-1-naphthaleneacetamide, urinary excretion accounted for 70.8-74.1% of the administered radioactivity suggesting that a multiple exposure regimen did not affect the absorption/excretion processes. Urinary excretion was unaffected following a single 100 mg/kg dose with 66.2-69.5% of the administered radioactivity excreted in urine. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (21.6-26.2%). Excretory patterns did not exhibit gender-related variability but reflected delayed absorption in the high-dose group. Because tissue burdens were very low at termination, neither 1-naphthaleneacetamide nor its metabolites appear to undergo significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC and HPLC/MS in conjunction with known standards. Urinary metabolism involved amide cleavage followed by glycine conjugation with the glycine conjugate being the major metabolite of the low and repeat doses (13.7-47.3% of the administered radioactivity). The glucuronide conjugate was also a major metabolite at the low doses (4.5-7.0% of administered). For feces, the major metabolite detected was the dihydrodiol of naphthaleneacetamide (3.6-11.3% of administered. Parent compound was detected at low concentrations (0.7-1.9% of administered) only in feces. Extraction efficiencies appeared to be excellent and most components in the matrices examined (urine and feces) were adequately quantified and characterized. The available data, based upon studies using labeled 1-naphthaleneacetamide, affirmed the metabolism pathway proposed by the investigators. This metabolism study is **Acceptable/Guideline** and satisfies the requirements for a tier 1 Metabolism and Pharmacokinetics Study.

B

Supplement to HED Document No. 005378 & 010667- DER for MRID No. 00108833 - Acute Oral Toxicity Study - Rat. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Abdallah Khasawinah, Ph.D. A. Khasawinah Date Sept. 4, 2003
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. W. Dykstra Date 10/10/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - [Rat] OPPTS 870.1100 [§81-1]; OECD 401.

DP BARCODE: D293238

P.C. CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthyl Acetamide (purity not provided, white powder)

SYNONYMS: Naphthalene Acetic Acid Acetamide, 1-NAA acetamide, 1-Naphthaleneacetamide

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Oral Toxicity Study in Rats (14 Day): Napthalene Acetamide: PH 402-UC-002-82. Pharmakon Research International, Inc. April 26, 1982. MRID 00108833. Unpublished.

SPONSOR: Union Carbide Agricultural Products Co., Inc., Research Triangle Park, NC

EXECUTIVE SUMMARY:

In an acute oral toxicity study (MRID 00108833), groups of Sprague Dawley rats (5/sex) were given 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) in 0.25% methylcellulose by single dose gavage at the following doses of 1000, 1500, 2000, 2500 or 3000 mg/kg bw. Dosage volume was 5 ml/rat for the two lowest dosages and 10 ml/rat for the three highest dosage levels. All animals were observed for up to 14 days post-dosing. Necropsy was performed on all animals.

The oral LD_{50} (95% C.I.) for males and females combined= 1690 mg/kg (1408-2028 mg/kg)

Signs of toxicity were convulsions, semi- prostration, piloerection, abnormal gait, abnormal stance, decreased activity and body tone, prostration, salivation, ptosis, tremors, hypersensitivity to touch, chromodacryarrhea, ataxia, body drop, vasoconstriction, and poor grooming. Necropsy

revealed stomach and intestines filled and distended of those animals dying during the study.

1-Naphthyl Acetamide is classified as **TOXICITY CATEGORY III**. This acute oral toxicity study in the rat was originally classified core-minimum (HED 005378) but a later review in 1993 (HED 010667) considered it supplementary due to lack of purity description in the study report and the use of excessive dosing volumes exceeding the guideline requirements not to exceed 1 ml/100 g bw. However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. The use of the excessive volume in dosing would exacerbate the toxicity of the test material rather than decrease it. Because of this later deficiency, the study is classified **Unacceptable/Guideline** and does not satisfy the OPPTS 870.1100 [§81-1]; OECD 401 requirement for Acute Oral Toxicity. A more recent **Acceptable/Guideline** study (MRID 43495901) satisfies this requirement.

COMPLIANCE: Signed and dated Quality Assurance statement provided. Confidentiality statements were not provided.

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

STUDY TYPE: ACUTE ORAL TOXICITY - RAT
[OPPTS 870.1100 (§81-1) OECD 401]
MRID 43495901

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

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

Primary Reviewer:
Susan Chang, M.S.

Signature: _____
Date: JUL 30 2003

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Date: JUL 30 2003

Robert H. Ross, M.S., Group Leader

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Date: JUL 30 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: JUL 30 2003

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: A. Khasawinah, Ph.D. **Signature:** A. Khasawinah
Reregistration Branch 4, Health Effects Division (7509C) **Date** August 27 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** W Dykstra
Reregistration Branch 4, Health Effects Division (7509C) **Date** 9/2/03
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/4/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat [OPPTS 870.1100 (§81-1) OECD 401].

PC CODE: 056001 **DP BARCODE:** D210844
SUBMISSION NO.: S315600, S508547

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetamide (98.7%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetamide - Acute oral toxicity study in rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar land, TX 77478. Laboratory study No. 1346-94, September 9, 1994. MRID 43495901. Unpublished. 13 pages.

SPONSOR: AMVAC Chemical Corporation, 2110 Davie Avenue, City of Commerce, CA 90040

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43495901), groups of fasted, young adult Sprague-Dawley rats (5/sex) were given a single oral dose of technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) at doses of 2000 or 5050 mg/kg bw by gavage. The test material was dosed as a 50% w/v mixture in 2% carboxymethyl cellulose (CMC) in deionized water and the animals were observed for 14 days.

One 5050 mg/kg male died during the study. Clinical signs including decreased activity, piloerection, lacrimation, ptosis, decreased defecation, gasping, polyuria, and/or ocular discharge were noted starting one hour after dosing with recovery by day 3 except for piloerection which cleared by day 10. With the exception of one 2000 female that lost weight during the first week and one 2000 mg/kg female and one 5050 mg/kg female that lost weight during the second week, all other surviving animals gained weight during the study. One 2000 mg/kg male had round gray foci in lungs and one 5050 mg/kg female had clear yellow-green liquid in the small intestine and an empty stomach. The decedent male had brick red and slightly swollen lungs, distended stomach with gas and a white liquid, and small intestines containing gas and a white paste.

Oral LD_{50} Males > 5050 mg/kg bw
Females > 5050 mg/kg bw

Combined > 5050 mg/kg bw

Technical 1-Naphthaleneacetamide is in EPA **Oral Toxicity Category IV.**

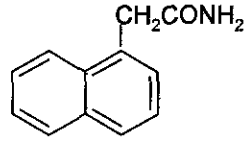
This acute oral toxicity study is classified as **Acceptable/Guideline.** This study satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Technical 1-naphthaleneacetamide

Description:	White powder
Lot/Batch #:	I 940415
Purity:	98.7% as reported in MRID 43896001 for this lot number
CAS # of TGAI:	86-86-2
Structure:	

NAA Acetamide

2. Vehicle and/or positive control: 2% w/v carboxymethyl cellulose (CMC) in deionized water as vehicle

3. Test animals:

Species:	Rat	
Strain:	HSD:Sprague-Dawley SD	
Age/weight at dosing:	Young adults; Males: 190-278 g, females: 198-225 g	
Source:	Harlan Sprague Dawley, Inc., Houston, TX	
Housing:	Individually in suspended stainless cage with wire bottom	
Diet:	Purina Formulab Chow No. 5008, *ad libitum* except for approximately 16 hours before dosing	
Water:	Municipal water, *ad libitum*	
Environmental conditions:	**Temperature:**	72±5°F
	Humidity:	30-80%
	Air changes:	10-12/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN AND METHODS:

1. In life dates: Start: July 6, 1994 (2000 mg/kg group) and July 13, 1994 (5050 mg/kg group); End: July 20, 1994 (2000 mg/kg group) and July 27, 1994 (5050 mg/kg group)

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of 2000 or 5050 mg/kg by gavage, observed at least three times after dosing, and once daily thereafter. The test material was dosed as a 50% w/v mixture in 2% carboxymethyl cellulose (CMC) in deionized water. The rats were weighed prior to dosing and on days 7 and 14 and at death. All surviving animals were sacrificed at the end of the study and a necropsy was performed.

TABLE 1. Doses, mortality/animals

Dose (mg/kg bw)	Males	Females	Combined
2000	0/5	0/5	0/10
5050	1/5	0/5	1/10

3. **Statistics:** Calculation of the oral LD_{50} was not needed.

II. RESULTS AND DISCUSSION:

A. **MORTALITY** is given in Table 1. One 5050 mg/kg male died on day 2.

The oral LD_{50} for males is > 5050 mg/kg,
females is > 5050 mg/kg, and
combined is > 5050 mg/kg.

B. **CLINICAL OBSERVATIONS:** Clinical signs including decreased activity, piloerection, lacrimation, and/or ptosis were noted in the 2000 mg/kg animals starting one hour after dosing with recovery by day 1, with the exception of piloerection on one male that recovered by day 5. In addition, decreased defecation was noted from the 5050 mg/kg males and gasping, polyuria, and/or ocular discharge were noted from one or two of the 5050 mg/kg females. All clinical signs disappeared in females by day 2 and in males by day 10.

C. **BODY WEIGHT:** One 2000 female lost weight during the first week and one 2000 mg/kg female and one 5050 mg/kg female lost weight during the second week. All other surviving animals had gained weight at the end of the study.

D. **NECROPSY:** One 2000 mg/kg male had round gray foci in the lungs and one 5050 mg/kg female had a clear yellow-green liquid in the small intestine and empty stomach. The decedent male had brick red and slightly swollen lungs, and a distended stomach with gas and a white liquid, and small intestines with gas and a white paste.

E. **REVIEWER'S CONCLUSION:** Technical 1-Naphthaleneacetamide is in EPA **Oral Toxicity Category IV.**

F. **DEFICIENCIES:** None

D

Supplement to HED Document No. 005378 & 010667- DER for MRID No. 00108832 - Acute Dermal Toxicity Study - Rabbit. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Abdallah Khasawinah, Ph.D. A. Khasawinah Date Sept. 4, 2003
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. W. Dykstra Date 10/10/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - [Rabbit] OPPTS 870.1200 [§81-2]; OECD 402.

DP BARCODE: D293238

P.C. CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthyl Acetamide (purity not provided, white powder)

SYNONYMS: Naphthalene Acetic Acid Acetamide, NAA Acetamide, 1-Naphthaleneacetamide

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Dermal Toxicity Test in Rabbits: NAA Acetamide: PH 422-UC-002-82. Pharmakon Research International, Inc. May 11, 1982. MRID 00108832. Unpublished.

SPONSOR: Union Carbide Agricultural Products Co., Inc., Research Triangle Park, NC

EXECUTIVE SUMMARY:

In an acute dermal toxicity study (MRID 00108832), five male and five female New Zealand White rabbits received 2 g/kg of 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) on the abraded skin under occlusive wrap for 24 hour exposure. Observations were made at 2, 4, 24 hour after exposure and twice daily thereafter for 14 days. Necropsy was performed on all animals. One animal died on day 11 with slight to moderate erythema and slight to moderate edema and skin scaling. The postmortem gross examination revealed brown foci on the lungs, discoloration of the intestines, heart and oral cavity. There was evidence of dermal irritation (erythema and edema) in all the animals. There were no treatment related gross necropsy changes in the animals that were sacrificed at the end of the study.

The dermal LD_{50} in the rabbit in this test is greater than 2 g/kg.

1-Naphthyl Acetamide is classified as **CATEGORY III for dermal toxicity**. This acute dermal toxicity study in the rabbit was originally classified core-minimum (HED 005378) but a later review in 1993 (HED 010667) considered it supplementary due to lack or reported compound purity in the study report and the use of abraded skin. However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. Although the use of abraded skin is not acceptable according to current standards of testing, the test material was moderately toxic under this extreme condition. Therefore the study is classified **Acceptable/Guideline** and satisfies the OPPTS 870.1200 [§81-2]; OECD 402 requirement for Acute Dermal Toxicity.

COMPLIANCE: Signed and dated Quality Assurance statement. Confidentiality statements were not provided.

E

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

STUDY TYPE: ACUTE DERMAL TOXICITY - RABBIT
[OPPTS 870.1200 (§81-2) OECD 402]
MRID 43495902

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

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

Primary Reviewer:
Susan Chang, M.S.

Signature: S Chang
Date: JUL 30 2003

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),Ph.D., D.A.B.T.

Signature: H.T Borges
Date: JUL 30 2003

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: JUL 30 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 30 2003

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: A. Khasawinah, Ph.D.
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D.
Reregistration Branch 4, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: A. Khasawinah
Date Aug. 27, 2003
Signature: W. Dykstra
Date 9/3/03
Signature: P.V. Shah
Date 9/8/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat [OPPTS 870.1200 (§81-2) OECD 402.

PC CODE: 056001

DP BARCODE: D210844
SUBMISSION NO.: S315600, S508547

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetamide (98.7%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetamide - Acute dermal toxicity study in rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar land, TX 77478. Laboratory study No. 1347-94, September 2, 1994. MRID 43495902. Unpublished. 13 pages.

SPONSOR: AMVAC Chemical Corporation, 2110 Davie Avenue, City of Commerce, CA 90040

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 43495902), five male and five female young adult New Zealand White rabbits were dermally exposed to a dose of 2020 mg/kg bw technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) moistened with saline. The treated area was wrapped with surgical gauze secured with non-irritating adhesive tape for 24 hours. Hair was clipped from the dorsal trunk and at least 10% of the body surface was exposed. The animals then were observed for 14 days.

Clinical signs including soft feces, diarrhea, and/or decreased defecation were noted on a few animals starting one hour post treatment with recovery by day 13. Erythema with or without edema was noted on four males and five females 1-2 days after patch removal with clearance by day 7. Desquamation was noted on two males and one female on days 1 or 10. With the exception of one male that lost weight during the first week, all animals gained weight during the study. Three males and two females had discolored surfaces on the livers, gastrointestinal tracts filled with gas, and/or a right kidney lighter in color. No observable abnormalities were noted from any other animal at necropsy.

Dermal LD_{50} **Males > 2020 mg/kg bw**
Females > 2020 mg/kg bw

Combined > 2020 mg/kg bw

Technical 1-Naphthaleneacetamide is in EPA **Dermal Toxicity Category III.**

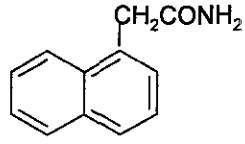
This acute dermal study is classified as **Acceptable/Guideline.** This study satisfies the guideline requirement for an acute dermal toxicity study (OPPTS 870.1200; OECD 402) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Technical 1-naphthaleneacetamide

Description:	White powder
Lot/Batch #:	I 940415
Purity:	Not reported.
CAS # of TGAI:	86-86-2
Structure:	

NAA Acetamide

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at dosing:	Young adults; Males: 2.300-2.925 g, females: 2.425-2.850 g	
Source:	Ray Nichols Rabbitry, Lumberton, TX	
Housing:	Individually in suspended stainless steel cage with wire bottom	
Diet:	Purina Rabbit Chow, in measured amount	
Water:	Drinking water from approximately 750 mL polycarbonates	
Environmental conditions:	**Temperature:**	72±5°F
	Humidity:	30-80%
	Air changes:	10-12/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN AND METHODS:

1. In life dates: Start: July 7, 1994; End: July 21, 1994

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Five male and five female animals were dermally exposed to 2020 mg/kg Technical 1-naphthaleneacetamide moistened with saline and applied to the clipped dorsal trunk (approximately 10% of the total body surface). The application site was covered with

surgical gauze and secured with non-irritating adhesive tape. The trunk was wrapped with a thin plastic film and secured in place with non-irritating adhesive tape. The covering was removed 24 hours after treatment and the treated area cleaned with tap water and cloth. The animals were observed at least three times post treatment, and at least once daily for 14 days. Body weight was recorded prior to treatment and on days 7 and 14. All animals were sacrificed and a necropsy was performed at the end of the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
2020	0/5	0/5	0/10

3. **Statistics:** Calculation of the dermal LD_{50} was not needed.

II. RESULTS AND DISCUSSION:

A. **MORTALITY** is given in Table 1. No animals died during the study.

The dermal LD_{50} for males is > 2020 mg/kg bw
females is > 2020 mg/kg bw
combined is > 2020 mg/kg bw

B. **CLINICAL OBSERVATIONS:** Soft feces was noted from four females on the day of treatment with recovery by day 1. Diarrhea was noted from one or more males on days 4 and 8-11 and one female on day 3. Decreased defecation was noted from a few animals on days 4 through 13. Erythema with or without edema was noted on four males and five females 1-2 days after patch removal with clearance by day 7. Desquamation was noted on two males and one female on days 1 or 10.

C. **BODY WEIGHT:** With the exception of one male that lost weight during the first week, all animals gained weight by the end of the study.

D. **NECROPSY:** Three males and two females had a discolored surface of the liver. In addition, one male and one female had gastrointestinal tracts filled with gas and one male had a right kidney lighter in color. No observable abnormalities were noted from any other animal.

E. **REVIEWER'S CONCLUSIONS:** Technical 1-naphthaleneacetamide is in EPA **Dermal Toxicity Category III**.

F. **DEFICIENCIES:** Individual clinical data were noted provided, but these would not change the results.

F

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT
[OPPTS 870.1300 (§81-3) OECD 403]
MRID 43495903

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

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: _____

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),Ph.D., D.A.B.T.

Signature: H.T. Borges
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: A. Khasawinah, Ph.D. **Signature:** A. Khasawinah
Reregistration Branch 4, Health Effects Division (7509C) **Date** August 27, 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** W Dykstra
Reregistration Branch 4, Health Effects Division (7509C) **Date** 9/2/03
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/8/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat [OPPTS 870.1300 (§81-3) OECD 403].

PC CODE: 056001 **DP BARCODE:** D210844
SUBMISSION NO.: S315600, S508547

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetamide (98.7%)

SYNONYMS: Not reported

CITATION: Holbert, M. (1994) Technical 1-Naphthaleneacetamide - Acute inhalation toxicity study in rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar land, TX 77478. Laboratory study No. 1348-94, October 13, 1994. MRID 43495903. Unpublished. 24 pages.

SPONSOR: AMVAC Chemical Corporation, 2110 Davie Avenue, City of Commerce, CA 90040

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 43495903), groups of young adult HSD:SD rats (5/sex/group) were exposed by whole body inhalation to 1-Naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) for 4 hours at analytical concentrations of 0.710 or 2.17 mg/L. The animals were then observed for 14 days.

Decreased activity, piloerection, and ptosis were noted from most of the animals during exposure. In addition, some 2.17 mg/L animals had a red crust around the eyes/nose starting on day 2. All clinical signs cleared by day 7. Two 0.710 mg/L females and two 2.17 mg/L females lost weight during the first week and one 2.27mg/L female lost weight during the second week. The other surviving animals gained weight during the study. The lungs of the decedent, one 0.710 mg/L female, and four 2.17 mg/L females were red and slightly swollen, possibly related to the administration of the test material.

Inhalation LC_{50} **Males > 2.17 mg/L**
Females > 2.17 mg/L
Combined > 2.17 mg/L

Technical 1-Naphthaleneacetamide is classified in EPA **Inhalation Toxicity Category IV.**

This acute inhalation study is classified as **Acceptable/Guideline.** This study satisfies the guideline requirement for an acute inhalation toxicity study (OPPTS 870.1300; OECD 403) in the rat.

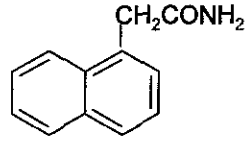
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Technical 1-naphthaleneacetamide

Description:	White powder
Lot/Batch #:	I 940415
Purity:	98.7% as reported in MRID 43896001 for this lot number.
CAS # of TGAI:	86-86-2
Structure:	



NAA Acetamide

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat	
Strain:	HSD:SD	
Age/weight at dosing:	Young adults; Males: 185-212 g, females: 196-234 g	
Source:	Harlan Sprague Dawley, Inc., Houston, TX	
Housing:	Individually in suspended stainless cage with wire bottom	
Diet:	Purina Formulab Chow No. 5008, *ad libitum* except during the exposure period	
Water:	Municipal water, *ad libitum* except during the exposure period	
Environmental conditions:	**Temperature:**	72±5°F
	Humidity:	30-80%
	Air changes:	10-12/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN AND METHODS:

1. In life dates: Start: August 23, 1994; End: September 7, 1994

2. Exposure conditions: Temperature and humidity were recorded at intervals of 30 minutes from a hygrometer located in the exposure chamber. The temperature and relative humidity of the chamber were 75-76°F and 82-83% humidity for the 0.710 mg/L group animals, and 74-76°F and 82-83% humidity for the 2.17 mg/L group animals.

3. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Rats were exposed to technical 1-naphthaleneacetamide by whole body exposure for four

hours. The animals were observed frequently on the day of exposure and at least once daily thereafter for 14 days. Only four males and three females could be observed during the 0.710 mg/L exposure due to chamber design and no 2.17 mg/L animals could be observed due to the test material covering the chamber window. Body weight was recorded prior to exposure and on days 7, 14, or at death. Survivors were sacrificed at the end of the study and a necropsy was performed on all animals.

TABLE 1. Concentrations, exposure conditions, mortality/animals treated

Nominal Conc. (mg/L)	Analytical Conc. (mg/L)	MMAD µm	GSD	Mortality (# dead/total)		
				Males	Females	Combined
8.83	0.710	5.147	2.410	0/5	0/5	0/10
40.9	2.17	4.826	2.248	0/5	1/5	1/10

4. **Generation of the test atmosphere / chamber description:** The aerosol was generated by a Venturi Aspirator which aspired the sifted test material with a motorized revolving disc delivery system coupled to the aspirator. The concentrated aerosol was elutriated through a 91 L baffling chamber and diluted to 0.710 mg/L with filtered air and drawn into the exposure chamber. The 2.17 mg/L level aerosol was generated with the same procedure without using the baffling chamber. Time to equilibrium was not reported. Analytical chemistry was conducted with a Bausch & Lomb Spectronic 2000 Spectrophotometer at 271 nm.

   Test atmosphere concentration: The exposure atmospheric samples were taken from the breathing zone of the animals once per hour during exposure. The atmospheric concentration was determined by analytical analysis. The nominal concentration was determined by dividing the loss in weight of the test material afer each exposure by the total volume of air which passed through the chamber. The average results are in Table 1 above.

   Particle size determination: The atmosphere samples were taken from the breathing zone twice during each exposure using an Anderson cascade impactor. The aerodynamic mass median diameter (MMAD) and geometric standard deviation (GSD) were then determined with the results in Table 1 above. The percent of total particles with sizes of ≤ 3.3 µm were 19% and 10-18% for the 0.710 and 2.17 mg/L groups, respectively.

5. **Statistics:** Calculation of the inhalation LC_{50} was not needed.

II. RESULTS AND DISCUSSION:

A. **MORTALITY** is given in Table 1. One female in the 2.17 mg/L group died on day 2.

The inhalation LC_{50} for males is > 2.17 mg/L,
females is > 2.17 mg/L,
combined is > 2.17 mg/L.

B. CLINICAL OBSERVATIONS: Decreased activity, piloerection, and ptosis were noted from most of the animals during exposure. In addition, some 2.17 mg/L animals had a red crust around eyes/nose starting day 2. All clinical signs recovered by day 7.

C. BODY WEIGHT: Two 0.710 mg/L females and two 2.17 mg/L females lost weight during the first week and one 2.27mg/L female lost weight during the second week. The other surviving animals gained weight during the study.

D. NECROPSY: The decedent female had red and slightly swollen lungs. One 0.710 mg/L female and four 2.17 mg/L females had mottled red and slightly swollen lungs. The study author stated that this was possibly related to the administration of the test material.

E. REVIEWER'S CONCLUSIONS: Technical 1-Naphthaleneacetamide is classified in EPA **Inhalation Toxicity Category IV.**

F. DEFICIENCIES: The MMAD in this study (5.147 and 4.826 μm) slightly exceeds the recommended MMAD range (1-4 μm), but would not significantly impact the results of the study.

G

Supplement to HED Document No. 005378 & 010667 - DER for MRID No. 00103051 - Primary Eye Irritation - Rabbit. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Abdallah Khasawinah, Ph.D. A. Khasawinah Date Sept. 4, 2003
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. W. Dykstra Date 10/10/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit; OPPTS 870.2400 [§81-4]; OECD 405.

DP BARCODE: D293238

P.C. CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthyl Acetamide (purity not provided, white powder)

SYNONYMS: NAA Acetamide, Naphthalene Acetic Acid Acetamide

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Eye Irritation Test in Rabbits: NAA Acetamide: Study No. PH 421-UC- 001-82. May 11, 1982. Pharmakon Research International, Inc. MRID 00103051. Unpublished.

SPONSOR: Union Carbide Agricultural Products Co., Inc., Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a primary eye irritation study (MRID 00103051), a dose of 100 mg of 1-Naphthyl Acetamide (white powder, Lot/Batch #: Not reported; Purity not reported) was instilled into the lower right eyelid of nine New Zealand albino rabbits. The eyes of three rabbits were washed immediately after treatment. The other six eyes were unwashed. Observations were made at 1, 2, 3, 4,7 10, and 13 days after treatment.

All of the animals showed signs of conjunctival irritation (redness, chemosis and/or discharge)that either partially or totally subsided over the course of the study. Three animals (two in the unrinsed group and one in the rinsed group) had corneal opacity that persisted in two animals (unrinsed group) through the end of the study. NAA Acetamide is **corrosive** for the eyes and is classified as **Category I** for eye irritation (HED 005378). However a later HED review in 1993 (HED 010667) did not assign a toxicity category because of a study deficiency in that the

observation period did not continue for 21 days as the guidelines require. For this reason and the lack of information on the purity of the test material and the lot used, the reviewers considered this study **Unacceptable**. However, the current reviewer considers the test material adequately described as white powder and the original HED review (HED # 005378) describes the active ingredient content of NAA acetamide as 97%. Therfore, the present reviewer, believes that in spite of these deficiencies, the study demonstrates the corrosiveness of the test material and the study is **acceptable/guideline** and it satisfies the guideline requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in the rabbit.

COMPLIANCE: Signed and dated Quality Assurance statement. Confidentiality statements were not provided.

H

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT
[OPPTS 870.2400 [§81-4]; OECD 405]
MRID 43495904

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

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

Primary Reviewer:
Susan Chang, M.S.

Signature: [signature]
Date: JUL 30 2003

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),Ph.D., D.A.B.T.

Signature: [signature]
Date: JUL 30 2003

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: JUL 30 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JUL 30 2003

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: A. Khasawinah, Ph.D. **Signature:** A. Khasawinah
Reregistration Branch 4, Health Effects Division (7509C) **Date** August 27, 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** W. Dykstra
Reregistration Branch 4, Health Effects Division (7509C) **Date** 9/3/03
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/8/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit [OPPTS 870.2400 (§81-4) OECD 405].

PC CODE: 056001 **DP BARCODE:** D210844
SUBMISSION NO.: S315600, S508547

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetamide (98.7%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetamide - Primary eye irritation study in rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar land, TX 77478. Laboratory study No. 1349-94, August 18, 1994. MRID 43495904. Unpublished. 17 pages.

SPONSOR: AMVAC Chemical Corporation, 2110 Davie Avenue, City of Commerce, CA 90040

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 43495904), 0.1 mL by volume (29 mg) of technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) was instilled into the conjunctival sac of the right eye of three male and three female young adult New Zealand White rabbits. The untreated eye served as a control. The animals then were observed for 72 hours.

Corneal opacity and iritis was not noted on any rabbit during the study. One male had positive conjunctival irritation (redness and chemosis) one hour after test material instillation with resolution by 24 hours. The highest maximum mean total score was 4.3 recorded one hour after test material instillation.

Technical 1-Naphthaleneacetamide was minimally irritating to the eye based on the highest maximum mean irritation total score (4.3) recorded one hour after test material instillation. Therefore, technical 1-Naphthaleneacetamide **is classified in EPA Toxicity Category IV** for eye irritation.

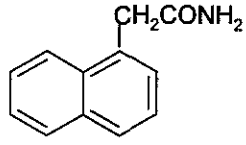
This study is classified as **Acceptable/Guideline**. This study satisfies the guideline requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Technical 1-naphthaleneacetamide

Description:	White powder
Lot/Batch #:	I 940415
Purity:	98.7% as reported in MRID 43896001 for this lot number
CAS # of TGAI:	86-86-2
Structure:	

NAA Acetamide

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at dosing:	Young adults; weight not reported	
Source:	Ray Nichols Rabbitry, Lumberton, TX	
Housing:	Individually in suspended stainless steel cage with wire bottom	
Diet:	Purina Rabbit Chow, in measured amount	
Water:	Drinking water from approximately 750 mL polycarbonates	
Environmental conditions:	**Temperature:**	72±5°F
	Humidity:	30-80%
	Air changes:	10-12/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN AND METHODS:

1. In life dates: Start: July 11, 1994; End: July 14, 1994

2. Animal assignment and treatment: The test material (29 mg) was instilled into the conjunctival sac of the right eye of three male and three female rabbits and the eye lids held together for approximately 1 second. The contralateral eye of all rabbits served as control. All eyes were washed with deionized water for one minute immediately after recording the 24-hour observation. The animals were scored for ocular irritation 1, 24, 48, and 72 hours after instillation.

II. RESULTS AND DISCUSSION:

A. Corneal opacity and iritis was not noted on any rabbit during the study. One male had positive conjunctival redness (grade 2) and chemosis (grade 2) one hour after test material instillation with resolution by 24 hours. The highest maximum mean irritation total score was 4.3 recorded one hour after test material instillation.

This classifies the test material as minimally irritating. Technical 1-naphthaleneacetamide is in TOXICITY CATEGORY IV.

B. **REVIEWER'S CONCLUSIONS:** Technical 1-Naphthaleneacetamide **is classified in EPA Toxicity Category IV** for eye irritation.

C. **DEFICIENCIES:** None

I

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG
[OPPTS 870.2600 (§81-6) OECD 406]
MRID 43495905

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

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

Primary Reviewer:
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: A. Khasawinah, Ph.D. **Signature:** A. Khasawinah
Reregistration Branch 4, Health Effects Division (7509C) **Date** August 27, 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** W. Dykstra
Reregistration Branch 4, Health Effects Division (7509C) **Date** 9/3/03
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 10/16/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Skin Sensitization - Guinea Pig [OPPTS 870.2600 (§81-6) OECD 406].

PC CODE: 056001 **DP BARCODE:** D210844
SUBMISSION NO.: S315600, S508547

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetamide (98.7%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetamide - Guinea pig maximization test for topically applied test material. Stillmeadow, Inc., 12852 Park One Drive, Sugar land, TX 77478. Laboratory study No. 1350-94, September 30, 1994. MRID 43495905. Unpublished. 19 pages.

SPONSOR: AMVAC Chemical Corporation, 2110 Davie Avenue, City of Commerce, CA 90040

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 43495905) with 5% v/v technical 1-naphthaleneacetamide (Lot No. I940415; purity 98.7% as reported in MRID 43896001 for this lot number) in cottonseed oil, 15 male and 15 female Hartley albino guinea pigs were tested using the Magnusson and Kligman test.

After the intradermal and topical inductions, no dermal reactions were noted from any animal following challenge.

In this study, Technical 1-Naphthaleneacetamide was not a dermal sensitizer.

The study was conducted in a manner suitable to detect the sensitization potential of the test material. The results of a positive control study performed within six months of the current study were not reported. In spite of this deficiency, this study is classified as **Acceptable /Guideline**. This study does satisfy the guideline requirement for a dermal sensitization study (OPPTS 870.2600; OECD 406) in the guinea pig.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical 1-naphthaleneacetamide

Description:	White powder
Lot/Batch #:	I 940415
Purity:	98.7% as reported in MRID 43896001 for this lot number.
CAS # of TGAI:	86-86-2

2. **Vehicle and/or positive control:** Cottonseed oil as the vehicle for intradermal injection and petroleum jelly for topical induction and challenge; results of a positive control were not included.

3. **Test animals:**

Species:	Guinea pigs	
Strain:	Hartley	
Age/weight at start:	Age not reported; males: 393-458 g; females: 387-460 g	
Source:	Harlan Sprague Dawley, Inc., Houston, TX	
Housing:	Individually in suspended stainless cage with wire bottom	
Diet:	Purina Guinea pig Chow, *ad libitum*	
Water:	Municipal water, *ad libitum*	
Environmental conditions:	**Temperature:**	72±5°F
	Humidity:	30-80%
	Air changes:	10-12/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN AND METHODS:

1. **In life dates:** Start: July 26, 1994; End: August 18, 1994

2. **Animal assignment and treatment:** The animals were induced and challenged according to the Magnusson-Kligman Maximization Test. The upper back across shoulders of 15 male and 15 female guinea pigs were clipped. Three pairs of intradermal injections (0.1 mL/site) were made into a 4 x 6 cm clipped area of skin on the back region of the test guinea pigs (10 males and 10 females) on day 0. The injectables were Freund's complete adjuvant (diluted to 50% v/v in 0.9% saline), 5% v/v test material in cottonseed oil, and 5% v/v test material in cottonseed oil in a 50:50 mixture of 50% v/v Freund's complete adjuvant in 0.9% saline. On day 6, the test sites were observed for irritation. On day 7, test material in petroleum jelly, saturated onto a 2 x 4 cm filter paper, was applied to the intradermal injection area under occlusion for 48 hours. The vehicle control animals (5 males and 5 females) were treated similarly to the test animals with the exception that the test material was omitted from the intradermal injections and topical application (with a patch dry filter paper). On day 21, the flanks of the test animals and the control animals were clipped. On day 22, the animals were topically challenged with 0.5 mL of a 50% w/v mixture of the test material in petroleum jelly and a patch of dry filter paper at naive sites on the right and left flanks for 24 hours. The sites were evaluated 24 and 48 hours post exposure.

II. RESULTS AND DISCUSSION:

A. INDUCTION REACTIONS AND DURATION: Erythema with or without edema was noted on all test animals. As for the control animals, erythema with or without edema was noted on the test sites injected with solution containing Freund's complete adjuvant, but not on the sites with vehicle control.

B. CHALLENGE REACTIONS AND DURATION: No dermal reaction was noted on any animal. Technical 1-naphthaleneacetamide was not a sensitizer.

C. POSITIVE CONTROL: No positive control study conducted within six months of the current study was reported.

D. REVIEWER'S CONCLUSIONS: It is the reviewer's opinion that the study was conducted in a manner suitable to detect the sensitization potential of the test material. Technical 1-naphthaleneacetamide was not a skin sensitizer in this test.

E. DEFICIENCIES: Results of a positive control study performed within six months of the current study was not provided.

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE (1-NAD)

Study Type: 82-1a; 90-Day Subchronic Oral Toxicity Study
of 1-Naphthaleneacetamide (1-NAD) in the Rat

Work Assignment No. 3-07A (MRID 43896001)

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

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
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Date: 9/14/97

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Date: _____

Quality Assurance
Reto Engler, Ph.D.

Signature: Reto Engler
Date: _____

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Abdallah Khasawinah, Ph.D. Date August 4, 2003
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. Date 8/4/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral FeedingToxicity - Rats; OPPTS 870.3100 [§82-1b] (rodents); OECD 408

DP BARCODE: D223223 **SUBMISSION CODE**: S5500658

P.C. CODE: 056001 **TOX. CHEM. NO.**: None

TEST MATERIAL (PURITY): 1-NAD (98.7% a.i.)

SYNONYMS: MRD-94-834; 1-Naphthaleneacetamide

CITATION: Trimmer, G.W. (1995) 90-Day subchronic oral toxicity study in the rat with 1-Naphthaleneacetamide (1-NAD). Exxon Biomedical Sciences, Inc., Toxicology Laboratory, Mettlers Road, CN 2350, East Millstone, NJ 08875-2350. Laboratory Project ID 183470B. 305 pages. November 15, 1995. MRID 43896001. Unpublished.

SPONSOR: Amvac Chemical Corporation, 4100 East Washington Boulevard, Los Angeles, CA 90023.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 43896001), 1-Naphthaleneacetamide (Lot # I940415; 98.7% a.i.) was administered to CRL:CD BR rats (10/sex/dose) by feeding at dose levels of 0, 250, 1,000, or 4,000 ppm (mean measured concentrations of 0, 19.1, 73.8, or 292.1 mg/kg/day for males and 0, 20.4, 81.5, or 313.5 mg/kg/day for females) for 90 days.

In the 4,000 ppm treatment groups, mean body weights were lower for males (10-15%) and females (9-12%) throughout the study, compared to controls. Final mean body weight gains were lower for males (14%) and females (20%). In addition, food consumption was consistently reduced for males (11-28%) and females (2-20%) throughout the study. Mean relative liver weights were significantly increased in both 4,000 ppm males (14%; $p \leq 0.05$) and females (32%; $p \leq 0.01$) with accompanying histopathological changes consisting of enlarged (hypertrophied) centrilobular hepatocytes with an abundance of fine granular eosinophilic cytoplasm. No rats died during the study. No treatment-related differences in clinical appearance, ophthalmology, hematology, clinical blood chemistry or urinalysis parameter or gross pathology were observed in any treatment group. No neoplastic tissue was observed in any of the treatment groups. The **LOAEL** is 4,000 ppm (292.1 mg/kg/day), based on decreased body weight, reduced body weight gain, reduced food consumption, and increased relative liver weights with histopathological changes in both sexes. The **NOAEL** is 1,000 ppm (73.8 mg/kg/day).

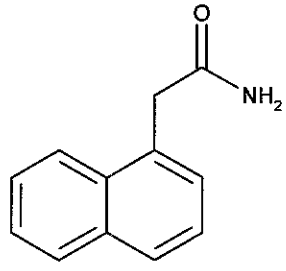
This 90-day oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodent species.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetamide (1-NAD)
   Description: White powder
   Lot/Batch #: I940415
   Purity: 98.7% a.i.
   Stability of compound: Shown to be stable for at least 14 days in feed stored at room temperature
   CAS #: 86-86-2
   Structure:



2. **Vehicle and/or positive control**: None

3. **Test animals**:
   Species: Rat
   Strain: CRL:CD BR
   Age and weight at study initiation: Approximately 5 weeks of age; body weight range - 122.9-146.6 g for males; 119.0-153.4 g for females
   Source: Charles River Laboratories, Inc., Kingston Facility; Stone Ridge, New York
   Housing: Individually housed in stainless steel and wire mesh cages
   Diet: PMI Certified Rodent Chow® #5002 (meal), ad libitum
   Water: Municipal tap water, ad libitum
   Environmental conditions:
      Temperature: 68-76^0 F
      Humidity: 40-70%
      Air changes: Not reported
      Photoperiod: 12 Hour light/12 hour dark cycle
   Acclimation period: 9 Days

B. STUDY DESIGN:

1. **In life dates** - Start: 8/18/94 End: 11/18/94

2. **Animal assignment**

   Animals (40/sex) were assigned to the test groups in Table 1 using a computer-generated, body weight sorting program.

Table 1. Study design.[a]

Test Group	Conc. in Diet (ppm)	Nominal Dose to Animals (mg/kg/day)[b]	Animals Assigned	
			Male	Female
Control	0	0	10	10
Low	250	25	10	10
Mid	1,000	100	10	10
High	4,000	400	10	10

[a] Dose selections were based on a 14-day palatability study conducted with 1-NAD (study not submitted with this MRID).

[b] Calculated by the reviewer using the Subdivision F Conversion Factor for a young rat weighing 0.1 kg

3. Dose selection rationale

Dose selections were based on a 14-day palatability study conducted with 1-NAD (study not submitted with this MRID).

4. Diet preparation and analysis

Diets were prepared fresh weekly by mixing appropriate amounts of 1-NAD with the feed and were stored at room temperature. The test material was incorporated into the feed at a fixed concentration, and was mixed thoroughly to ensure homogeneity.

Prior to the initiation of dosing, samples were collected from the top, middle, and bottom of the 250 and 4,000 ppm diet preparations for homogeneity analyses. Samples were analyzed in triplicate to confirm adequacy of mixing. Additional samples were collected from the middle of the two diet preparations for stability analyses. Two samples were taken; one was stored under laboratory conditions at room temperature and the other stored frozen. Analyses were conducted at 0, 4, 8, and 14 days of storage. Concentration analyses were performed for each diet on Weeks 1, 5, 9, and 13. If batch sizes varied by more than 30% from previous batches, homogeneity analyses were repeated.

Results

Homogeneity: The concentration of 1-NAD in the feeds was:
250 mg/kg/day: 95.6-98.4% of nominal
4,000 mg/kg/day: 93.3-96.4% of nominal

Stability analysis (concentration after 14 days):
Room temperature:
250 mg/kg/day: 98.8% of nominal
4,000 mg/kg/day: 97.1% of nominal

Frozen:
250 mg/kg/day: 95.9% of nominal

4,000 mg/kg/day: 100.7% of nominal

Concentration analysis (1, 5, 9, and 13 weeks):
250 mg/kg/day: 90.0-97.2% of nominal
1,000 mg/kg/day: 95.7-99.4% of nominal
4,000 mg/kg/day: 94.6-98.3% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Statistical evaluation of the equality of means was conducted using one-way analysis of variance (ANOVA) and a test for ordered response in the dose groups. Bartlett's test was initially performed to determine if the dose groups had equal variance. If the variances were equal, parametric methods were used; otherwise, nonparametric methods were employed. For the parametric procedures, a standard one-way ANOVA using the F distribution to assess significance was used. If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed significantly from the control. A standard regression analysis for linear response in the dose groups was also performed. The regression also tested for linear lack of fit in the model. For the nonparametric procedures, the Kruskal-Wallis Test was used to test for the equality of means. If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control. Jonckheere's Test for monotonic trend in the dose response was also performed. Bartlett's Test for equal variance was conducted at the 1% level of significance. All other statistical tests were conducted at the 1 and 5% levels of significance.

C. METHODS:

1. Observations:

a. Cageside Observations - All animals were observed for viability twice daily during the weekdays and once daily on weekend days and holidays.

b. Clinical Observations - Clinical observations were made daily for signs of toxicity.

c. Neurological Evaluations - These were limited to clinical signs of toxicity.

2. Body weight

Animals were weighed prior to the initiation of dosing for group allocation, on the day of dosing (Day 0), weekly during the study, on the day of fasting, and at terminal sacrifice.

3. Food consumption and compound intake

Food consumption (g) for each animal was determined weekly during the treatment period. Mean food consumption for each test group was calculated weekly during the study period; for males, the Week 13 value was a 6-day value due to fasting on Day 90 for blood collection on Day 91. Food efficiency (g bw/g food consumed) was calculated weekly during the test period for all animals based on food consumption, the number of days in the sampling interval, and

the average body weight gain during the interval. Mean compound intake values (mg/kg/day) were calculated as time-weighted averages from the consumption and weight gain data.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed on both eyes of each test animal using focal illumination and indirect ophthalmoscopy prior to dose initiation and just prior to study termination. Mydriasis was induced with 1% atropine sulfate.

5. Hematology & Clinical Chemistry:

Blood was collected following overnight fasting from the abdominal aorta of all males on Day 91 and all females on Day 92 for hematology and clinical analyses. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic toxicity studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES	X	Triglycerides
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
	Gamma glutamyl transferase (GGT)		
X	Gamma glutamyl transpeptidase		

* Required for subchronic toxicity studies.

6. Urinalysis

Urine was collected from all test animals at the termination of the inlife phase of the study. The CHECKED (X) parameters were examined.

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

7. Sacrifice and Pathology

All test animals were sacrificed by exsanguination following methoxyflurane anesthesia at study termination and were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow (sternum)*	X	Spinal cord (3 levels)*
X	Stomach*				
X	Duodenum*	X	Lymph nodes*	X	Pituitary*
X	Jejunum*	X	Spleen*	X	Eyes (optic n.)*
X	Ileum*	X	Thymus*		
X	Cecum*				
X	Colon*				GLANDULAR
X	Rectum*		UROGENITAL		
XX	Liver*+	XX	Kidneys*+		
X	Pancreas*	X	Urinary bladder*	XX	Adrenal gland*
		XX	Testes*+	X	Harderian gland
		X	Epididymides	X	Lacrimal gland[T]
	RESPIRATORY	X	Prostate	X	Mammary gland[T]
		X	Seminal vesicles	X	Thyroid with parathyroids*+
X	Trachea*	XX	Ovaries*+		
X	Lung*	X	Oviducts		
	Nose	X	Uterus*		OTHER
	Pharynx				
	Larynx			X	Bone (femur and sternum)*
				X	Skeletal muscle*
				X	Skin*
				X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic toxicity studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Mortality - No rats died during the study.

2. Clinical Signs - No treatment-related abnormalities in appearance or behavior were noted in any of the treatment groups during the study. All clinical signs of toxicity occurred randomly and sporadically in all treatment and control groups.

3. Neurological Evaluations - See clinical signs above.

B. BODY WEIGHT AND WEIGHT GAIN

At study termination, body weight gain was reduced for both males (14%) and females (20%) in the 4,000 ppm treatment group (Table 2). Although the overall weight gain reduction for the entire 90-day feeding period was not statistically significant for either sex, the mean body weights were significantly less than the controls for males during Weeks 1-9 ($p \leq 0.05$ or 0.01) and for females during Weeks 1-3 and Week 8 ($p \leq 0.05$). In addition, the mean body weights and mean body weight gains were consistently reduced for the majority of the study, compared to controls. These findings were considered treatment-related. In the 1,000 ppm treatment groups, a transient reduction in body weight gain in both sexes only during weeks 1 and/or 2, while statistically significant, was not considered treatment-related. No differences in body weights and body weight gains were noted in the 250 ppm treatment groups compared to the controls.

Table 2. Average body weights and body weight gains for male and female rats.[a]

Conc. in Diet (ppm)	Body Weight (g)		Body Weight Gain[c] (g)			Mean Final Body Wt. Change (% of Control)[c]
	0 Days	90 Days[b]	0-28 Days	0-49 Days	0-90 Days[b]	
Males						
0	126.3	525.8	220.9	310.5	399.5	---
250	126.3	541.3	227.9	312.0	415.0	+3.9
1,000	125.7	521.2	202.6	292.3	395.5	-1.0
4,000	125.5	469.1	181.0	260.4	343.6	-14.0
Females						
0	127.0	302.9	104.6	143.5	175.9	---
250	127.0	310.8	104.2	144.8	183.8	+4.5
1,000	126.9	310.1	103.5	145.1	183.2	+4.2
4,000	126.5	267.1	83.3	115.9	140.6	-20.1

[a] Data obtained from Tables 2 and 3, pages 52-55, in the study report.
[b] Final body weights for female rats were recorded on Day 91 of the study.
[c] Calculated by the reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - In the 4,000 ppm treatment groups, food consumption was consistently lower than controls throughout the study for males (11-28%) and females (2-20%). Mean food consumption was significantly lower ($p \leq 0.05$ or 0.01) for males during Weeks 1-5, 7, 9, and 13, and for females during Weeks 1, 8, 12, and 13 compared to controls. Food consumption for the 250 and 1,000 ppm treatment groups was, in general, comparable to the controls.

2. Compound consumption - The average consumption of 1-NAD by male and female rats is presented in Table 3. Measured compound consumption by male rats in the 250, 1,000, and 4,000 ppm treatment groups averaged 19.1, 73.8, and 292.1 mg/kg/day, respectively, over the 13-week treatment period. Measured compound consumption by females in the 250, 1,000, and 4,000 ppm treatment groups averaged 20.4, 81.5, and 313.5 mg/kg/day, respectively. During the study, male and female rats ingested 48-140% of the nominal dose of 1-NAD.

Table 3. Average consumption of 1-NAD by rats during 90-day feeding study with 1-NAD.[a]

Test Group	Conc. in Diet (ppm)	Nominal Dose to Animal (mg/kg/day)	Actual Dose (mg/kg/day)	
			Male [mean]	Female [mean]
Control	0	0	0	0
Low	250	25	35-13 [19.1]	32-16 [20.4]
Mid	1,000	100	124-50 [73.8]	128-62 [81.5]
High	4,000	400	454-194 [292.1]	426-236 [313.5]

[a] Data extracted from table on page 29 of the study report.

3. Food efficiency - Mean food conversion efficiency for both sexes in the 4,000 ppm treatment groups during week 1 was 31-32% compared to controls. In general, the mean food conversion efficiency of the 4,000 ppm group females was decreased throughout most of the study. In the 4,000 ppm group males, there were significant increases and decreases in mean food conversion efficiency throughout the study, but overall, the conversion efficiency was similar to the controls. No treatment-related differences in food consumption were noted for the 250 and 1,000 ppm treatment groups compared to controls.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ophthalmoscopic findings were observed at any treatment level.

E. BLOOD ANALYSIS

1. Hematology - No treatment-related differences in hematology parameters were observed in any of the treatment groups. Decreased prothrombin time in the 4,000 ppm group females (8%; $p \leq 0.01$) was not clearly treatment-related since there was no clear consistent pattern of response or similar findings in activated thromboplastin time.

2. Clinical Chemistry - No treatment-related effects in clinical blood chemistry parameters were observed in any of the treatment groups compared to controls. Decreased chloride levels in the 1,000 ppm group females and increased calcium levels in the 4,000 ppm group females, while statistically significant ($p \leq 0.05$ or ≤ 0.01), differed by $< 5\%$ of the control values, and therefore, were biologically insignificant. Cholesterol levels were increased but not statistically significant in the 1-NAD treated males and were considered not treatment related. Increased cholesterol levels in females were also increased but statistically significant only in the 4000 ppm group (59% increase; $p \leq 0.01$). The increase in the low and mid dose females was 42 and 17%, respectively, suggesting a lack of a treatment-related trend.

F. URINALYSIS - No treatment-related effects were observed in urine parameters of rats in any of the treatment groups. Increased urine protein in the 1,000 ppm group females lacked a clear dose-relationship, and increased urine glucose in the 1,000 and 4,000 ppm females were not considered clinically significant, although they were statistically significant compared to the controls.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight - Relative liver weights for the 4,000 ppm group males and females wre 14 and 32% higher ($p \leq 0.05$ or 0.01) compared to the controls (Table 4). Absolute liver weights for the females only were 16% ($p \leq 0.05$) higher than controls in the 4,000 ppm group. Increased relative testes weight in the 4,000 ppm group males (22%; $p \leq 0.01$) was attributed to the low mean body weight; no associated macroscopic findings were observed.

Table 4. Absolute and relative liver weights of rats after 90 days of treatment.[a]

Conc. in Diet (ppm)	Terminal Body Weight[b] (g)	Liver	
		Absolute (g)	Relative
Male			
0	493.8	13.87	0.028
250	510.0	14.56	0.028
1,000	490.9	13.80	0.028
4,000	439.2	14.04	0.032*
Female			
0	283.2	7.10	0.025
250	289.7	7.77	0.027
1,000	291.3	7.95	0.027
4,000	248.7*	8.24*	0.033**

[a] Data were obtained from Table 12 and 13, pages 77 and 78 of the study report.
[b] Body weight at sacrifice.
* Significantly different from controls, $p \leq 0.05$.
** Significantly different from controls, $p \leq 0.01$.

2. Gross pathology - No treatment-related differences in gross pathology were observed between rats in the treatment and control groups.

3. Microscopic pathology

a. Non-neoplastic - The livers of 1/10 males in the 1,000 ppm treatment group and 5/10 males and 8/10 females in the 4,000 ppm treatment group exhibited hypertrophied centrilobular hepatocytes with an abundance of fine granular eosinophilic cytoplasm. No other treatment-related postmortem differences were observed between rats in the treatment and control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

b. Neoplastic - No neoplastic tissue was observed in rats in the treatment or control groups.

III. DISCUSSION AND CONCLUSIONS

A. Investigator's Conclusions

The study author concluded that male and female rats fed 1-NAD in the diet at 4,000 ppm for 90 days exhibited decreased body weights, suppressed body weight gains, and reduced food consumption compared to controls. Adaptive treatment-related changes were observed in the livers of both sexes, primarily at the 4,000 ppm dose level, which consisted of increased relative liver weights and enlarged centrilobular hepatocytes with an abundance of eosinophilic cytoplasm. In the absence of any other histopathological findings, these adaptive changes were not considered to be toxicologically significant. Therefore, the NOAEL was established at 1,000 ppm. Based on the study author's conclusions, the LOAEL for this study would be 4,000 ppm.

C. Reviewer's Discussion

We agree with the study author's findings that treatment-related effects observed at 4,000 ppm treatment level were decreased body weights and body weight gains and reduced food consumption compared to controls. At study termination, body weight gain was reduced for both males (14.0%) and females (20%). Food consumption was consistently lower than controls throughout the study in both males (11-28%) and females (2-20%). Mean food consumption was statistically significantly less than controls during Weeks 1-5, 7, 9, and 13 in males ($p \leq 0.05$ or 0.01) and during Weeks 1, 8, 12, and 13 in females ($p \leq 0.05$ or 0.01). Food consumption in the 250 and 1,000 ppm groups were comparable to controls.

At the 4,000 ppm treatment level, relative liver weights were higher in both males (14%; $p \leq 0.05$) and females (32%; $p \leq 0.01$), compared to controls. The absolute liver weights were significantly higher than controls in 4,000 ppm females (16%; $p \leq 0.05$), but not in males. In males (5/10) and females (8/10) treated at 4,000 ppm, microscopic changes in liver were observed which were characterized by enlarged (hypertrophied) centrilobular hepatocytes with an abundance of eosinophilic cytoplasm. Because other histopathologic findings were absent, these were considered, by the study author, to be adaptive changes in the liver associated with an increase in liver weight and, therefore, of no toxicological significance. However, because relative liver weights were significantly increased and there were associated histopathological changes in both sexes at 4,000 ppm, we must disagree and conclude that these changes are both treatment-related and toxicologically significant. Additionally, the blood cholesterol levels were highly elevated in the 4000 ppm females.

At 1,000 and 250 ppm, there were no significant differences in absolute or relative liver weights for either sex. Histopathological changes consisting of hypertrophied centrilobular hepatocytes were observed in only on 1,000 ppm male and were not considered to be of toxicological significance.

No rats died during the treatment period. No treatment-related differences in clinical appearance, ophthalmology, hematology, clinical blood chemistry, or urinalysis parameters or gross pathology were observed between rats in the treatment and control groups. No neoplastic tissue was observed in any of the treatment groups.

The **LOAEL** for this study is 4,000 ppm (292.1 mg/kg/day) based on lower body weight, reduction in body weight gain, reduced food consumption, and increased relative liver weights with histopathological changes. The **NOAEL** is 1,000 ppm (73.8 mg/kg/day).

Based on the weight gain reductions observed in this 90-day study, the dose selection for a chronic study should include a 300 mg/kg/day level (4000 ppm). In the absence of other significant toxicological effects, this dose does not seem excessive.

B. STUDY DEFICIENCIES

No deficiencies were noted in this study.

DATA FOR ENTRY INTO ISIS

Subchronic (90-day) Oral Toxicity Study- rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
056001	43896001	subchronic	rat	90 days	oral	dietary	0-4000 ppm	males:19, 74, 292 Females: 20, 82, 314	73.8	292.1	Body weight, liver	Systemic

K

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE (1-NAD)

Study Type: 82-1a; 90-Day Subchronic Oral Toxicity Study
of 1-Naphthaleneacetamide (1-NAD) in the Dog

Work Assignment No. 3-07B (MRID 43895901)

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

Prepared by
Pesticides Health Effects Group
Sciences Division
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Date: 6/19/03

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Abdallah Khasawinah, Ph.D. A. Khasawinah Date Aug. 1, 2003
Reregistration Branch 4, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. William Dykstra Date 8/4/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [capsule] - dogs; OPPTS 870.3150 [§82-1b] (non-rodent); OECD 409

DP BARCODE: D223223 **SUBMISSION CODE**: S5500658

P.C. CODE: 056001 **TOX. CHEM. NO.**: None

TEST MATERIAL (PURITY): 1-NAD (98.7% a.i.)

SYNONYMS: MRD-94-834; 1-Naphthaleneacetamide

CITATION: Farrell, D. (1995) A 13-week subchronic oral (capsule) toxicity study of 1-Naphthaleneacetamide (1-NAD) in the beagle dog. Bio-Research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, Canada, H9X 3R3. Laboratory Project ID 86451. 340 pages. October 20, 1995. MRID 43895901. Unpublished.

SPONSOR: Amvac Chemical Corporation, 4100 East Washington Boulevard, Los Angeles, CA 90023.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 43895901), 1-NAD (Lot/Batch # I940415; 98.7% a.i.) was administered via capsule to four beagle dogs/sex/dose at dose levels of 0, 30, 100, or 300 mg/kg/day for 13 weeks.

In the 300 mg/kg/day treatment group, all livers contained accumulations of a hemosiderin-containing pigment in the reticuloendothelial cells and bilirubin in the intracanicular spaces. The spleens of 3/4 males and 2/4 females also contained hemosiderin and hematopoiesis was increased in the bone marrow in 3/4 animals of both sexes. Decreases in red blood cell counts, hematocrit, and hemoglobin occurred in both sexes. Platelet counts and mean corpuscular volumes were increased in both sexes. Total bilirubin was increased in 1/4 males and 3/4 females, but the increases were significant ($p<0.05$ or 0.01) only for females. Body weights were reduced in males only. Clinical signs of toxicity in both sexes were soft or liquid feces. No treatment-related effects were observed in the 30 or 100 mg/kg/day treatment groups.

No dogs died during the study. No treatment-related differences in clinical appearance, food consumption, ophthalmology, urinalysis parameters, organ weights, or gross pathology were observed in any treatment group. No neoplastic tissue was observed in any of the treatment groups. **The LOAEL is 300 mg/kg/day, based on increased platelet count, decreased red cell parameters, and increased mean corpuscular volume which correlate with**

histopathological changes observed in the liver, spleen, and bone marrow in both sexes. The NOAEL is 100 mg/kg/day.

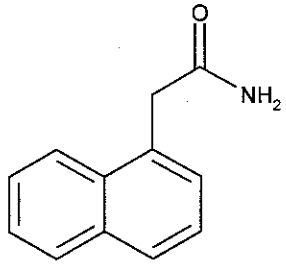
This 90-day oral toxicity study in the dog is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in non-rodent species.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetamide (1-NAD)
   Description: White powder
   Lot/Batch #: I940415
   Purity: 98.7% a.i.
   Stability of compound: Not described, test article was stored at circa 4°C when not in use
   CAS #: 86-86-2
   Structure:



2. **Vehicle and/or positive control**: None

3. **Test animals**:
   Species: Dog
   Strain: Beagle
   Age and weight at study initiation: Approximately 4 months of age; body weight range - 8.0-9.9 kg for males; 7.0-8.9 kg for females
   Source: HRP Inc., 6321 South 6th Street, Kalamazoo, MI
   Housing: Individually housed in stainless steel cages with a bar type floor
   Diet: PMI Certified Laboratory Chow® #5007, approximately 400 g daily
   Water: Municipal tap water, filtered (reverse osmosis) and UV sterilized, ad libitum
   Environmental conditions:
      Temperature: 20±3 C
      Humidity: 50±20%
      Air changes: Not reported
      Photoperiod: 12 Hour light/12 hour dark cycle
   Acclimation period: 33 Days

B. STUDY DESIGN:

1. **In life dates** - Start: 1/30/95 End: 5/2/95

2. **Animal assignment**: Animals (16/sex) were assigned to the test groups in Table 1 by weight using a computer-generated randomization program.

Table 1. Study design.

Test Group	Dose to Animals (mg/kg/day	Animals Assigned	
		Male	Female
1 Control	0	4	4
2 Low	30	4	4
3 Mid	100	4	4
4 High	300	4	4

3. Dose selection rationale

Dose selection was based on two previous studies (not included with current submission) in which 1-NAD was administered as a single dose at 100, 200, or 400 mg/kg/day or for 14 days at 150 or 250 mg/kg/day. Rats treated with a single dose at 200 or 400 mg/kg/day had red material in the stools. Females treated with repeated doses at 250 mg/kg/day exhibited dark discoloration of the digesta and dark foci in the pyloric region of the stomach. No other treatment-related gross pathological changes were observed.

4. Diet Preparation and Analysis

Doses were prepared daily and were based on the most recently recorded weekly body weights. The test article was dispensed into size 11 gelatin capsules that were then stored at room temperature in the dark until use. Storage stability was determined by distribution of a 3 g sample into the appropriate number of size 11 gelatin capsules which were then stored refrigerated for 7 days. The capsule contents were then analyzed for stability. The results were not included in the study report.

4. Statistics

Means and standard deviations were derived for the numerical data obtained during the study. The data were analyzed for homogeneity of variances using Bartlett's test. Homogeneous (parametric) data were analyzed using Analysis of Variance (ANOVA) and the significance of inter-group differences was assessed using Dunnett's t-test. Heterogeneous (nonparametric) data were analyzed using the Kruskal-Wallis test and the significance of the intergroup differences was assessed using Dunn's test.

C. METHODS:

1. Observations:

All animals were observed twice daily for viability and clinical signs of toxicity. Each animal was given a weekly physical examination.

2. Body weight:

Animals were weighed twice prior to the initiation of dosing and weekly during the treatment period. Fasted body weights were measured just prior to sacrifice.

3. Food consumption:

Food consumption (g) for each animal was measured daily beginning two weeks prior to treatment and continuing throughout the treatment period. Mean food consumption for each treatment group was calculated weekly during the study period.

4. Ophthalmoscopic examination:

Ophthalmological examinations were performed on all test animals prior to start of treatment and on all surviving animals during Week 12 using funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examination procedures. Mydriacyl (1%) was used to dilate the pupils.

5. Hematology & Clinical Chemistry:

Blood was collected following overnight fasting during Weeks 4, 8, and 12 for hematology and clinical analyses. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*	X	Red cell distribution width
	(Thromboplastin time)	X	Blood cell morphology
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic toxicity studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulin (calculated)
X	Sodium*	X	A/G ratio
		X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
		X	Triglycerides
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Gamma glutamyl transpeptidase		

* Required for subchronic toxicity studies.

6. Urinalysis

Urine was collected from all test animals during Weeks 4, 8, and 12 following overnight deprivation of food and water. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Occult Blood
X	Sediment (microscopic)	X	Nitrite
X	Protein	X	Urobilinogen

7. Sacrifice and Pathology:

All test animals were sacrificed by exsanguination following sodium pentobarbital anesthesia at study termination and were subject to external morphological and gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow (sternum)*	X	Spinal cord (3 levels)*
X	Stomach*				
X	Duodenum*	X	Lymph nodes*	XX	Pituitary*
X	Jejunum*	XX	Spleen*	X	Eyes (optic n.)*
X	Ileum*	X	Thymus*		
X	Cecum*				
X	Colon*				GLANDULAR
X	Rectum*		UROGENITAL		
XX	Liver*+	XX	Kidneys*+		
X	Gallbladder	X	Urinary bladder*	XX	Adrenal gland*
X	Pancreas*	XX	Testes with epididymides*+		Harderian gland
				X	Lacrimal gland[T]
		X	Prostate	X	Mammary gland[T]
	RESPIRATORY		Seminal vesicles	XX	Thyroid with parathyroids*+
		XX	Ovaries*+		
X	Trachea*		Oviducts		
X	Lung*	X	Uterus*		OTHER
	Nose				
	Pharynx			X	Bone (femur and sternum)
	Larynx				
X	Tonsils			X	Skeletal muscle*
				X	Skin*
				X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic toxicity studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. Mortality - No dogs died during the study.

2. Clinical Signs -

In the 300 mg/kg/day treatment group, treatment-related clinical signs of toxicity observed were soft/liquid or black feces (25-100% of total amount). Soft/liquid feces occurred throughout the treatment period in both sexes, but was more pronounced in 3-4/4 males and 2-4/4 females during the first six weeks of treatment. Instances of soft/liquid feces occurred on 100 days total in 4/4 males and 51 days total in 4/4 females at 300 mg/kg/day. There were 4 days total in which black feces were observed after dosing in 2/4 males and no instances of black feces in females after dosing at 300 mg/kg/day. The changes in fecal consistency reported in other treatment groups were random and sporadic and not treatment-related. No other treatment-related differences in clinical appearance were observed in any of the treatment groups.

3. Neurological Evaluations - See clinical signs above.

B. Body weight and weight gain

At study termination, body weight gain was reduced for males (25%) but not females (3%) in the 300 mg/kg/day treatment group (Table 2). There were no treatment-related differences in body weight gain at 30 or 100 mg/kg/day.

Table 2. Body weight changes in dogs after 13 weeks of treatment with 1-NAD.[a]

Dose Level (mg/kg/day)	Group Mean Body Weight Gains (kg)			
	Males[b]	% of Control[c]	Females[b]	% of Control[c]
0	2.33	0	1.93	0
30	2.22	-5	2.15	+12
100	3.12	+34	2.60	+35
300	1.75	-25	1.87	-3

[a] Data were obtained from Tables 2 and 3, pages 35-37 and summarized on page 23 of the study report.
[b] Mean total weight gained during 13 weeks of feeding.
[c] % change relative to control.

C. Food consumption

No treatment-related differences in mean food consumption were observed in any treatment group.

D. Ophthalmoscopic examination

No treatment-related ophthalmoscopic findings were observed in any treatment group.

E. BLOOD ANALYSIS:

1. Hematology -

Hematology results are summarized in Tables 3 and 4 for male and female dogs, respectively. At 300 mg/kg/day, treatment-related decreases in red blood cell counts were observed during Weeks 4, 8, and 12 in males (19-24%, $p<0.01$) and during Weeks 4 and 12 in females (22-23%, $p<0.01$); hemoglobin values were significantly lower than controls in males (16-19%) during Weeks 8 and 12 ($p<0.05$ and 0.01, respectively) and females (18%) during Week 4 ($p<0.05$); and hematocrit values were significantly lower in males (19%, $p<0.01$) and females (18%, $p<0.05$) during Week 12. Also at 300 mg/kg/day of 1-NAD, significant increases in mean corpuscular volume were observed in males (8-10%) during Weeks 4, 8, and 12 ($p<0.01$) and females (10%) during Week 4 ($p<0.05$). Platelets were significantly increased at 300 mg/kg/day for both males (32-56%) and females (82-83%) during Weeks 8 and 12 ($p<0.05$ and 0.01, respectively. In the 100 mg/kg/day males, the isolated decrease in RBC counts (12%, $p<0.05$) during week 12 was not considered treatment-related. No treatment-related effects were observed in the 30 mg/kg/day treatment groups.

Table 3. Levels of affected hematology parameters in male dogs at intervals during 13 weeks of dosing with 1-NAD.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	30	100	300
Red Blood Cells (x 10^6/µL)				
Pretest	6.44	6.18	5.93	6.19
4	6.44	6.63	6.14	5.19**
8	6.71	6.98	6.38	5.37**
12	7.36	7.14	6.51*	5.57**
Hemoglobin (g/dL)				
Pretest	14.1	13.7	13.4	13.9
4	14.1	14.8	14.0	12.2
8	14.8	15.6	14.6	12.5*
12	16.0	15.9	14.8	12.9**
Hematocrit (%)				
Pretest	40.8	39.6	38.9	40.o
4	40.8	42.4	40.3	36.1
8	42.5	44.8	41.9	36.6
12	47.0	46.0	43.2	38.2**
Mean Corpuscular Volume (µm^3)				
Pretest	63.3	64.4	65.5	64.7
4	63.3	64.2	65.6	69.5**
8	63.2	64.2	65.7	68.2**
12	63.8	64.5	66.4	68.6**
Platelets (x 10^3/µl)				
Pretest	373.0	367.5	338.5	385.5
4	319.8	298.8	305.8	393.5
8	331.8	340.8	335.5	439.3*
12	236.8	271.3	295.0	370.5**

[a] Data obtained from Table 5, pages 39-62 of the study report.
* Significantly different from controls, $p<0.05$.
** Significantly different from controls, $p<0.01$.

Table 4. Levels of affected hematology parameters in female dogs at intervals during 13 weeks of dosing with 1-NAD.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	30	100	300
Red Blood Cells (x 10^6/µL)				
Pretest	6.47	6.04	6.14	6.20
4	6.90	6.38	6.60	5.38**
8	6.87	6.39	6.66	5.62
12	7.14	6.95	6.72	5.51**
Hemoglobin (g/dL)				
Pretest	14.8	13.6	13.9	14.1
4	15.9	14.5	15.2	13.0*
8	15.8	14.8	15.5	13.5
12	16.4	16.1	15.7	13.4
Hematocrit (%)				
Pretest	42.6	39.5	40.1	40.6
4	45.5	42.1	43.6	38.8
8	45.2	42.2	44.1	39.5
12	47.3	46.0	44.9	39.0*
Mean Corpuscular Volume (μm^3)				
Pretest	65.9	65.3	65.4	65.6
4	66.0	66.1	66.1	72.4*
8	65.8	66.1	66.2	70.7
12	66.3	66.3	66.7	71.1
Platelets (x 10^3/µL)				
Pretest	351.5	392.5	348.0	395.8
4	285.3	316.0	332.5	347.3
8	231.8	331.5	306.5	422.0*
12	218.5	276.3	258.5	400.3**

[a] Data were obtained from Table 5, pages 39-62 of the study report.
* Significantly different from controls, $p<0.05$.
** Significantly different from controls, $p<0.01$.

2. Clinical Chemistry -

At 300 mg/kg/day, bilirubin values for both males (1/4) and females (3/4) were increased during Weeks 4, 8, and 12, however the increases were only statistically significant in females at 4 ($p\leq0.01$) and 12 ($p\leq0.05$) weeks (↑80 and 136%, respectively). The increased bilirubin values for 300 mg/kg/day males at 12 weeks (↑31%) was due to one very high value and one somewhat high value compared to controls. There were no other treatment-related effects at 300 mg/kg/day and no treatment-related effects on clinical chemistry parameters at 30 or 100 mg/kg/day.

F. URINALYSIS - No treatment-related effects were observed in urine parameters of dogs in any of the treatment groups.

G. SACRIFICE AND PATHOLOGY:

1. Organ weights -

No treatment-related differences in organ weights were observed in any treatment groups. Differences in absolute or relative organ weights that were statistically significant were random and sporadic and were not considered to be biologically significant or treatment-related.

2. Gross pathology - No treatment-related differences in gross pathology were observed between dogs in the treatment and control groups.

3. Microscopic pathology

a. Non-neoplastic - The livers of all 300 mg/kg/day animals (4/sex) contained an accumulation of a yellowish brown pigment, present primarily in reticuloendothelial cells, which tested positive for hemosiderin. A similar pigment in the intracanalicular spaces tested positive for bilirubin and suggests a hemolytic effect of the test copound. Additionally, the spleens of 3/4 males and 2/4 females in the 300 mg/kg/day treatment group contained a pigment testing positive for hemosiderin. An increase in hematopoiesis was observed in the bone marrow of 3/4 of the 300 mg/kg/day males and females.

No treatment-related histopathological changes were observed in dogs in the 30 and 100 mg/kg/day treatment groups compared to controls.

b. Neoplastic - No neoplastic tissue was observed in dogs in the treatment or control groups.

III. DISCUSSION & CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that male and female dogs fed 1-NAD in the diet at 300 mg/kg/day for 13 weeks exhibited toxicity to the hematopoietic system. Body weight gain was reduced in males. Clinical signs of toxicity included soft, liquid or black feces in both sexes, increases in total bilirubin in females, and increases in platelet counts and decreases in red blood cell parameters (red blood cell count, hemoglobin, and hematocrit) in both sexes. Hemosiderin accumulation was observed in the liver and spleen and there was increased hematopoietic activity in the bone marrow of both sexes. The **NOAEL** for this study was identified as 100 mg/kg/day.

B. REVIEWER'S DISCUSSION

Although we agree with the study author's observations concerning blood cell parameters and correlated histopathological findings, we disagree that the results observed are evidence of hematopoietic system toxicity. There is an abnormal bone marrow involvement which can be characterized as systemic toxicity. ~~We believe that the increased hematopoietic activity observed in bone marrow smears is not evidence of hematopoietic system toxicity, but rather that the system is attempting to compensate for the effects of macrocytic, hypochromic anemia by negative feedback homeostasis mechanisms. These mechanisms would result in an increase in~~

L

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE

Study Type: 82-2; 21-Day Repeated Dose Dermal Toxicity Study in the Rat

Dynamac Study No. 1-31A (MRID 43581001)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Quality Assurance:
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Signature:
Date: 4/16/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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RAB3, Health Effects Division (7509C) **Date** August 1, 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** William Dykstra
RRB4, Health Effects Division (7509C) **Date** 8/6/03

Template version 11/01

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - Rat ; OPPTS 870.3200 [§82-2] (rodent); OECD 410.

DP BARCODE: D213980 **SUBMISSION NO.**: S484981

PC CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthaleneacetamide Technical (98.71%)

SYNONYM(S): Rootone, 1-NAD; MRD-94-834

CITATION: Trimmer, G.W. (1995) 21-Day Repeated Dose Dermal Toxicity Study in the Rat with 1-Naphthaleneacetamide (MRD-94-834). Exxon Biomedical Sciences, Inc., Toxicology Laboratory, Mettlers Road, East Millstone, NJ, 08875-2350. Laboratory Project ID 183410B. 145 pages. March 2, 1995. MRID 43581001. Unpublished.

SPONSOR: AMVAC Chemical Corporation, Los Angeles, CA.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 43581001), 1-Naphthaleneacetamide (Lot # I940415; 98.71% ai) was applied to the shaved skin of Crl:CD BR rats (5/sex/dose) at dose levels of 0, 100, 300, or 1000 mg/kg for 6-6.5 hours/day, 5 days/week, for 3 weeks. No treatment-related effects were observed at any dose level. There were no clinical signs of toxicity, and body weights, body weight gains, and food consumption were similar between the treated and control groups. No differences were observed in hematology parameters or clinical blood chemistry. Skin irritations occurred at similar rates in rats in all groups. Although males in the 1000 mg/kg treatment group had absolute liver weight which was 16.4% heavier ($p < 0.05$) than the control, no accompanying anatomical or functional changes were observed, and the mean liver weights of females in the 1000 mg/kg treatment group were lower than the control. No other differences in the organ weights, and no differences in macroscopic or microscopic organ morphology were observed between rats in the treated and the control groups. No neoplastic tissue was observed at any dose level. Ophthalmoscopic examinations and urinalysis were not performed during the study. No **LOAEL** was established. The **NOAEL** was the highest treatment level, 1000 mg/kg body weight.

This 21-day dermal toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 21-day dermal toxicity study (OPPTS 870.3200 ; OECD 410) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

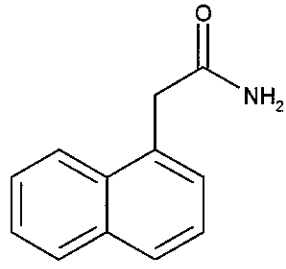
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 1-Naphthaleneacetamide (1-NAD)

Description: White powder
Lot/Batch #: I 940415
Purity: 98.7% ai
Stability of compound: The test material was shown to be stable at room temperature for the duration of the study.

CAS #: 86-86-2
Structure:



2. Vehicle and/or positive control: None

3. Test animals:

Species: Rat
Strain: Crl:CD BR
Age and weight at study initiation: Males - approximately 8 weeks of age with a body weight range of 248-291 g; Females - approximately 10 weeks, 222-256 g
Source: Charles River Laboratories, Kingston Facility, Stone Ridge, NY
Housing: Individually housed in elevated stainless steel, wire mesh cages
Diet: PMI Feeds, Certified Lab Chow #5002 (Mash), ad libitum
Water: Municipal tap water, ac libitum
Environmental conditions:
- Temperature: 68-76° F
- Humidity: 40-70%
- Air Changes: Not specified
- Photoperiod: 12-Hour light/dark cycle

Acclimation period: 13 Days

B. STUDY DESIGN:

1. In life dates - Start: 8/22/94 End: 9/12/94

2. Animal assignment

Rats (20/sex) were selected for use on the basis of pretest examinations which excluded animals in poor health, or with outlying body weights or other abnormalities. The selected rats were assigned to the test groups in Table 1 using a computer generated sorting program.

TABLE 1: STUDY DESIGN.

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1 Control	0	5	5
2 Low	100	5	5
3 Mid	300	5	5
4 High	1000	5	5

3. Dose selection rationale

No explanation was provided for the selected dose levels.

4. Preparation and treatment of animal skin

Approximately 24 hours before the initial exposure, and weekly thereafter, the fur on each rat was "closely clipped" on the dorsal surface from the shoulder region to the lumbar region using electric clippers, so that approximately 10% of the body surface was exposed. A measured amount of 1-naphthaleneacetamide was moistened by placing reverse osmosis water (1 mL/g test material) on a gauze patch. The patch was then placed over a 60 x 40 mm area on the clipped skin, and the animal was wrapped with COBAN. The rats were exposed to the test compound for 6 to 6.5 hours/day, 5 days each week, for 3 weeks. After each exposure, the dressings were removed and the residual compound was removed with reverse osmosis water.

Rats in the control group were exposed to reverse osmosis water (2 mL/kg body weight) only, but otherwise handled as described for the treated animals.

5. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. If the variances were found to be equal, the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test. If variances proved to be unequal, the data were analyzed by the Kruskal-Wallis test followed by Dunn's summed rank test. Trends related to the dose level were analyzed using either standard regression techniques with a test for trend and lack of fit, or by Jonckheere's test for monotonic trend to determine significance. Bartlett's test was conducted at the 1% level of significance; all other tests were conducted at the 5% and 1% levels.

C. METHODS:

1. Observations

1a. Cageside Observations - Animals were observed twice daily on weekdays and once daily on weekends for "viability".

1b. Clinical Examinations - Clinical examinations were conducted twice daily during the week and once daily on weekends for signs of toxicity. The rats were evaluated for dermal irritation on days 0, 1, 4, 7, 11, 14, and 18, and on the day of sacrifice using the Draize method.

1c. Neurological Evaluations - No neurological evaluations were conducted.

2. Body weight

Animals were weighed during the week prior to the initial dosing,. prior to dosing on days 0, 7, and 14, prior to fasting on day 20, and on the day of sacrifice.

3. Food consumption

Food consumption for each animal was determined for the 0- to 7-, 8- to 14-, and 15- to 20-day periods (7, 7, and 6 day totals). Animals were fasted on day 20. Mean diet consumption was reported as g food/week, and was not adjusted for body weights.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

5. Hematology & Clinical Chemistry:

Blood was collected from all rats on day 21. Animals were fasted overnight prior to the collection of blood from the abdominal aorta while under methoxyflurane anesthesia. The CHECKED (X) parameters were examined in all samples analyzed. (Although slides were prepared to determine reticulocyte counts, this parameter was not measured because "other RBC parameters were normal".)

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium* (K)		Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. Urinalysis

Urine was not collected during the study. It is optional for a 21/28 day dermal study.

7. Sacrifice and Pathology

All animals were sacrificed at the termination of the study and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination; however, only the skin (treated and untreated sites), kidneys, liver, ovaries or testes, and all tissues with gross lesions were actually examined. The XX organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	X	Brain*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
				X	Harderian gland
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder* (not rat)	X	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated areas)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		
X	Lung*		Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required.

II. RESULTS

A. OBSERVATIONS:

1. Clinical Signs of toxicity - No rats exhibited obvious treatment related abnormalities during the study.

2. Mortality - No rats died during the study.

3. Neurological Evaluations - No neurological evaluations were conducted.

4. Dermal Irritation - Desquamation was observed in three 300 mg/kg males (day 11), and in two or three 1000 mg/kg males (days 4, 7, and 11). Very slight erythema was observed in one 100 mg/kg female (days 18 and 21), and one 300 mg/kg female (day 21).

B. BODY WEIGHT AND WEIGHT GAIN:

There were no significant differences between the terminal body weights and body weight gains of rats in the treated and control groups. On day 20 (prior to fasting), the average weight of male rats was 372-386 g, and of female rats was 269-278 g (data from Table 4 of the study report).

C. FOOD CONSUMPTION AND EFFICIENCY:

Food consumption by the treated and control groups was similar.

D. OPHTHALMOSCOPIC EXAMINATION

Ophthalmoscopic examinations were not performed during the study.

E. BLOOD ANALYSIS:

1. Hematology - No treatment-related differences were observed between hematology parameters of rats in the treated and control groups. All parameters remained within expected ranges.

2. Clinical Chemistry - No treatment-related differences were observed between the clinical blood chemistry of rats in the treated and control groups. All parameters remained within expected ranges.

F. URINALYSIS:

Urine was not analyzed during the study.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight - Males in the 1000 mg/kg treatment group had significantly higher absolute and relative liver weights ($p < 0.05$ and 0.01, respectively) than the controls (Table 2).

TABLE 2. ABSOLUTE AND RELATIVE LIVER WEIGHTS OF MALE RATS.[a]

	Dose level (mg/kg)			
Observation	0	100	300	1000
Absolute liver weight (g)	10.53	10.73	10.73	12.26*
Relative liver weight	0.031	0.032	0.032	0.035**

[a] Data obtained from Tables 11 and 12, pages 49 and 50, in the study report.
* Significantly different from the control, $p < 0.05$.
** Significantly different from the control, $p < 0.01$.

Although the mean absolute kidney weight of males in the 100 mg/kg treatment group was significantly ($p < 0.05$) higher than the controls, this was primarily due to the presence of one low-kidney-weight animal in the control group and one high-kidney-weight animal in the 100 mg/kg group. If these outliers (2.54 and 3.28 g, respectively) are disregarded, the absolute kidney weights of the remaining 18 male rats ranged from 2.72 to 3.17 g. Relative kidney weights were similar for all groups. Thus the results at the 100 mg/kg/day are not dose-related. No other differences in the absolute or relative organ weights were observed between rats in the treated and the control groups.

2. Gross pathology - No treatment-related gross postmortem differences were observed between rats in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic -No treatment-related microscopic differences were observed between rats in the treated and the control groups. Skin irritations such as hyperplasia, hyperkeratosis, inflammation were observed at similar rates in skin from the clipped areas of rats in all groups, and were considered to be a result of the clipping and/or wrapping of the area.

b) Neoplastic - No neoplastic tissue was observed in rats in the treatment and control groups.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that the **NOAEL** of 1naphthaleneacetamide was 1000 mg/kg for rats under the conditions of this study. The basis of this decision was the lack of "signs of overt toxicity" in all dose groups.

B. REVIEWER'S DISCUSSION:

We agree with the study author's conclusions that the **NOAEL** of 1-naphthaleneacetamide was 1000 mg/kg. 1-Naphthaleneacetamide had no effect on male and female rats at any treatment level. There were no clinical signs of toxicity, and body weights, body weight gains, and food consumption were similar between the treated and control groups. No differences were observed in hematology parameters or clinical blood chemistry. Skin irritations occurred at similar rates in rats in all groups, and were considered to be a result of the clipping and/or wrapping of the treated area. Although males in the 1000 mg/kg treatment group had livers 16.4% heavier ($p < 0.05$) than the control, no accompanying anatomical or functional changes were observed, and the mean liver weights of females in the 1000 mg/kg treatment group were lower than the control. No other differences in the organ weights, or macroscopic or microscopic morphology were observed between rats in the treated and the control groups. No neoplastic tissue was observed in rats in the treatment and control groups. Ophthalmoscopic examinations and urinalysis were not performed during the study. No **LOAEL** was established. The **NOAEL** was the highest treatment level, 1000 mg/kg body weight which is also the limit dose.

IV. STUDY DEFICIENCIES:

No deficiencies were noted in the study.

DATA FOR ENTRY INTO ISIS

Subchronic Dermal (28 day) Study - rodents (870.3200)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
056001	43581001	subchronic	rat		dermal	dermal	0-1000	0, 100, 300, 1000	1000	NA	none	

M

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch, Health Effects Division (7509C)
EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D.
RRB4, Health Effects Division (7509C)

Signature: Nancy E. McCarroll
Date 08/06/03
Signature: A. Khasawinah
Date Aug. 7, 2003

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro*** **Bacterial Gene Mutation** *Salmonella typhimurium*/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471

DP BARCODE: D214872 **SUBMISSION NO.**: S484982

PC CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthaleneacetamide Technical (98.7%)

SYNONYM(S): Rootone

CITATION: San, R.H.C. and M.L. Klug (1995) Salmonella Plate Incorporation Mutagenicity Assay (Ames Test) With a Confirmatory Assay; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU54.501001; Study Completion Date: February 6, 1995. MRID: 43581006. Unpublished

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In a microbial/mammalian microsome plate incorporation mutagenicity study (MRID No: 43581006), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to five doses of 1-naphthalene- acetamide technical (Lot # I 940415; 98.7%) ranging from 100 to 5000 µg/plate with and without S9 activation. Two independent trials were conducted. The S9 homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The test material was delivered to the test system in dimethyl sulfoxide.

Compound insolubility was seen at ≥3333 µg/plate +/-S9. Cytotoxicity was observed for all strains at 5000 µg/plate +/-S9 and for the majority of strains at 3333 µg/plate -S9. All strains responded in the expected manner to the appropriate positive control. **There was, however, no indication that 1-naphthaleneacetamide technical induced a mutagenic effect at any dose with or without S9 activation.**

This study is classified as **Acceptable/Guideline**, and satisfies the guideline requirement for the

requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetamide technical
   Description: White powder
   Lot/batch number: I 940415
   Purity: 98.7%
   Receipt date: June 10, 1994
   Stability: Expiration date: May 1, 1998
   CAS number: 86-86-2
   Structure: Not provided
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The test material was stored at room temperature, protected from light and moisture. Dosing solutions were adjusted to 100% active ingredient and were analyzed for actual concentration.

2. **Control Materials**:

   Negative: None
   Solvent/final concentration: 50 µL DMSO/plate
   Positive: Nonactivation:

Sodium azide	1.0 µg/plate TA100, TA1535
2-Nitrofluorene	1.0 µg/plate TA98, TA1538
9-Aminoacridine	75 µg/plate TA1537
Other:	None

   Activation:

2-Aminoanthracene	1.0 µg/plate all strains.

3. **Activation**: S9 derived from male Sprague-Dawley

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	noninduced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

The rat liver S9 homogenate was prepared by the performing laboratory and was characterized for its ability to metabolize 7,12-dimethylbenzanthracene and 2-aminoanthracene to mutagens prior to use.

S9 mix composition	Final concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10 %

4. Test Organism Used: S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		list any others		

Properly maintained?	[X]	Yes	[ ]	No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	[X]	Yes	[ ]	No

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg/plate) were evaluated with or without S9 activation in S. typhimurium strain TA100. Single plates were used per dose per condition.

(b) Mutation assays:

Initial Trial: Five doses (33, 100, 333, 1000 and 5000 µg/plate) were evaluated in all tester strains in both the presence and the absence of S9 activation. Triplicate plates were prepared per dose per strain per condition.

Confirmatory Trial: As above for the initial trial.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:

_X_ standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other *(describe)*

2. **Protocol**:

(a) Plating procedures: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays.

One hundred µL of an overnight broth culture of the appropriate tester strain and 50 µL of the appropriate test material dose, solvent, or positive controls and either 0.5 mL sham cofactor mix (nonactivated tests) or 0.5 mL of the S9 mix were added to tubes containing 2.0 mL of top agar. The contents of the tubes were mixed, poured over minimal medium, and incubated at 37°C ± 2°C for ≈48-72 hours. At the end of incubation, plates were either immediately scored for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations were determined for the mutation assay.

(b) Sterility controls: A sterility test was performed on the highest dose of the test material and 0.5 mL of the sham cofactor or S9 mix as described for the mutation assay.

3. **Statistical Analysis**: None described

4. **Evaluation criteria**:

a. **Assay validity**: The assay was considered valid if the following criteria were met: (1) the presence of the appropriate genetic markers was verified for each strain; (2) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; (3) cell densities were $\geq 0.3 \times 10^9$ cells/ml; and (4) all positive controls caused at least a 3-fold increase in revertants per plate compared to the respective solvent control.

b. **Positive response**: The test material was considered positive if it caused a ≥2-fold increase in mean revertant colonies of strains TA98 and TA100, or if it caused a ≥3-fold increase in mean revertant colonies of strains TA1535, TA1537, or TA1538 and the increase was accompanied by a dose-response to increasing concentrations of the test material.

II.. REPORTED RESULTS:

A. **Analytical Determinations**: Data presented from the analytical determinations of test material concentrations prepared for both trials of the mutagenicity assay indicated that dosing solutions were within 86.5% of the intended concentrations for the initial trial and within 91.9% of the target levels for the confirmatory trial.

B. **Preliminary Cytotoxicity Assay**: Ten doses ranging from 6.7 to 5000 µg/plate +/-S9

were assayed for cytotoxic effects on strain TA100. Moderate compound precipitation and an ≈56% reduction in histidine revertants (his^+) was observed at the high dose with and without S9 activation. Compound insolubility but no cytotoxicity was seen at 3333 µg/plate +/-S9. Based on these findings, the initial mutation assay was conducted with five doses ranging from 100 to 5,000 µg/plate +/-S9.

C. **Mutation Assays**: Representative results from the initial and confirmatory assays are presented in Study Report Tables 22 and 23, pp 37 and 38; respectively (see attachment). In agreement with the preliminary results, 1-naphthaleneacetamide technical was insoluble at the two highest doses (3333 and 5000 µg/plate +/-S9). Data from the two independent trials were also in good agreement and indicated that the test material was cytotoxic for all strains at 5000 µg/plate +/-S9 and for the majority of strains at 3333 µg/plate -S9. No appreciable increases in his^+ revertant colonies were, however, noted at any noncytotoxic level of the nonactivated or S9-activated test substance. By contrast, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive control in both trials.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS**: Based on the overall results, the study authors concluded that 1-naphthalene-acetamide technical was negative in this microbial test system.

B. **REVIEWER'S DISCUSSION/CONCLUSIONS**: We assess that the study authors interpreted the data correctly. The test material was evaluated up to insoluble levels (≥3333 µg/plate +/-S9), was cytotoxic for all strains at the highest investigated dose (5000 µg/plate +/-S9) and for the majority of strains at nonactivated 3333 µg/plate but failed to induce a mutagenic effect. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. It was, therefore, concluded that the study provided acceptable evidence that 1-naphthaleneacetamide technical was negative in this bacterial gene mutation assay.

This study is classified as **Acceptable/Guideline**, and satisfies the guideline requirement for the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. **STUDY DEFICIENCIES**: Although analytical determinations for the high dosing solution (initial trial) indicated an ≈14% difference between actual and target concentrations, this deficiency did not alter the outcome of the study. Sufficient indicators of cytotoxicity were demonstrated at the highest dose tested, hence, the study would have been acceptable even without analytical determinations. The requirement that actual concentrations in dosing solutions be within 10% of the intended levels is, therefore, waived.

Attachment 1. Reproduced from study report MRID 43581006, page37

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article ID :Technical 1-NAPHTHALENEACETAMIDE
Study Number :G94AU54.501001 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation					
Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
Liver Microsomes : None					
0.0	26 ± 5	141 ± 20	13 ± 3	6 ± 1	8 ± 2
100	28 ± 5	123 ± 8	11 ± 4	7 ± 3	11 ± 3
333	29 ± 4	139 ± 5	12 ± 3	7 ± 1	9 ± 2
1000	23 ± 5	125 ± 11	10 ± 1	4 ± 3	8 ± 2
3333	17 ± 3	89 ± 7	4 ± 2	4 ± 1	6 ± 1
5000	12 ± 3	70 ± 9	5 ± 3	4 ± 2	7 ± 2
Pos	252 ± 13	896 ± 86	701 ± 18	1302 ± 154	367 ± 45
Liver Microsomes: Rat Liver S9					
0.0	37 ± 4	173 ± 10	9 ± 2	8 ± 4	14 ± 1
100	35 ± 4	162 ± 7	13 ± 4	7 ± 4	16 ± 4
333	35 ± 4	151 ± 9	13 ± 2	6 ± 3	17 ± 1
1000	34 ± 2	136 ± 7	12 ± 2	7 ± 3	16 ± 1
3333	27 ± 6	104 ± 18	12 ± 4	4 ± 2	11 ± 4
5000	23 ± 4	93 ± 16	7 ± 2	4 ± 2	8 ± 2
Pos	904 ± 128	555 ± 33	111 ± 6	125 ± 18	1379 ± 41

0.0 = Vehicle plating aliquot of 50 µl
Pos = Positive control concentrations as specified in Materials and Methods section

Attachment 1. Reproduced from study report MRID 43581006, page 38

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article ID :Technical 1-NAPHTHALENEACETAMIDE
Study Number :G94AU54.501001 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation					
Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
Liver Microsomes : None					
0.0	36 ± 8	129 ± 9	14 ± 1	6 ± 1	8 ± 3
100	2 ± 7	135 ± 19	9 ± 1	3 ± 3	7 ± 1
333	25 ± 4	122 ± 26	9 ± 1	2 ± 2	8 ± 2
1000	23 ± 6	117 ± 2	13 ± 5	3 ± 2	10 ± 1
3333	12 ± 4	71 ± 11	4 ± 1	1 ± 1	5 ± 1
5000	8 ± 2	47 ± 9	3 ± 1	1 ± 2	5 ± 2
Pos	264 ± 8	650 ± 19	491 ± 36	472 ± 99	393 ± 29
Liver Microsomes: Rat Liver S9					
0.0	22 ± 3	142 ± 7	12 ± 5	5 ± 2	12 ± 1
100	33 ± 6	138 ± 12	15 ± 4	5 ± 2	13 ± 6
333	29 ± 3	143 ± 13	12 ± 2	7 ± 4	17 ± 2
1000	19 ± 7	134 ± 4	10 ± 1	5 ± 3	14 ± 1
3333	17 ± 5	86 ± 2	4 ± 2	5 ± 2	11 ± 2
5000	12 ± 5	68 ± 15	3 ± 1	1 ± 2	8 ± 4
Pos	451 ± 64	2013 ± 95	161 ± 25	304 ± 79	1161 ± 322

0.0 = Vehicle plating aliquot of 50 µl
Pos = Positive control concentrations as specified in Materials and Methods section

N

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Signature: A. Khasawinah
Date Aug. 7, 2003

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice, OPPT 870.5395/ (§84-2) OECD 474

DPBARCODE: D214872 **SUBMISSION NO.**: S484982

PC CODE: 056001 **TOX. CHEM. NO.**: 588

TEST MATERIAL (PURITY): 1-Naphthaleneacetamide Technical (98.7%)

SYNONYM(S): Rootone

CITATION: Putman, D.L. and R.R. Young (1994) Micronucleus Cytogenetic Assay in Mice; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU54.122; 36 pages. Study Completion Date: November 30, 1994. MRID 43581005. Unpublished

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No: 43581005), groups of five male and five female ICR mice received single intraperitoneal injections of 250, 500 or 1000 mg/kg 1-naphthaleneacetamide technical (Lot # I940415; 98.7%). The test material was delivered to the animals as suspensions prepared in 1% aqueous carboxymethylcellulose. Animals were sacrificed at 24, 48 or 72 hours postexposure and bone marrow cells were harvested and examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

Overt toxicity in high-dose animals included death and lethargy. Slightly depressed polychromatic to normochromatic erythrocyte ratios (PCE:NCE) were also observed in both sexes of the high-dose group. The positive control induced the expected high yield of MPEs in males and females. There was, however, no indication that 1-naphthaleneacetamide technical induced a clastogenic or aneugenic effect in either sex at any dose or sacrifice time.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetamide technical

Description: White powder
Lot/batch number: I 940415
Purity: 98.7%
Receipt date: June 10, 1994
Stability: Expiration date: May 1, 1998
CAS number: 86-86-2
Structure: Not provided
Vehicle used: 1% Aqueous carboxymethylcellulose (CMC)
Other provided information: The test material was stored at room temperature, protected from light and moisture. Dosing suspensions were prepared on the day of use, adjusted to 100% active ingredient and analyzed for actual concentration.

2. **Control Materials**:

Negative Control /Route of administration: None

Vehicle/Final concentration/Route of administration: 1% CMC was administered by intraperitoneal (IP) injection at a dosing volume of 20 mL/kg.

Positive Control /Final concentration/Route of administration: Cyclophosphamide (CP) was dissolved in distilled water at a concen-tration of 2 mg/mL and was administered IP at 40 mg/kg.

3. **Test Animals**:

Species:	Mouse
Strain:	ICR
Age/weight at study initiation:	6-8 weeks; • Pilot study: 28.2-35.6 g (males); 27.4-31.6 g (females) • Toxicity test Trial I: 30.9–35.8 g (males); 25.7–30.4 g (females) • Toxicity test Trial II: 33.0-37.9 g (males); 24.9-30.8 g (females) • Micronucleus assay: 27.8-36.3 g (males); 23.7-32.1 g (females)
Source:	Harlan Sprague Dawley, Inc., Frederick, MD
No. animals used per dose	See Section A 4 (a,b,c,d)
Properly Maintained?	Yes

NOTE: All animals were weighed immediately before compound administration; dosing was based on individual body weights.

4. **Test Compound:**

Route of administration: IP

Dose levels used:

(a) Pilot study: 1, 10, 100 and 1000 mg/kg (2 males/dose)
5000 mg/kg (5 males and 5 females)

Note: The dosing volume for all levels except the high dose was 20 mL/kg. Due to solubility problems, the highest dosing concentration that could be prepared was 125 mg/kg; therefore, to achieve a high dose of 5000 mg/kg, the dosing volume was increased to 40 mL/kg.

(b) Toxicity test Trial I: 800, 1400, 2200 and 3500 mg/kg
(5 males and 5 females/dose)

(c) Toxicity test Trial II: 1000 and 1200 mg/kg
(5 males and 5 females/dose)

(d) Micronucleus assay: 250, 500 and 1000 mg/kg
(5/animals/sex/dose/sacrifice time)

Secondary group: An additional group of 5 males and 5 females received the high dose of the test material and were used to replace animals that died in the primary high-dose group.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound and vehicle control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	X	24 hr	X	48 hr	X	72 hr
Other : None										

b. Positive control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	X	24 hr		48 hr		72 hr
Other : None										

2. **Tissues and Cells Examined**:

Bone marrow OR other :	Bone marrow
No. of polychromatic erythrocytes (PCE) examined per animal:	1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Scored/1000 PCEs
Other (if other cell types examined, describe):	None

3. **Details of Slide Preparation**: At 24, 48, and 72 hours after administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the positive control group was 24 hours. Bone marrow cells were aspirated from both femurs into fetal bovine serum, centrifuged, resuspended and spread onto slides. Prepared slides were fixed in methanol, stained with May-Gruenwald-Giemsa solution and coverslipped. Slides were coded prior to scoring.

4. **Evaluation Criteria**:

   a. **Assay validity**: The study was considered valid if the mean number of micronucleated polychromatic erythrocytes (MPEs) in the negative (vehicle) control did not exceed 0.5%, and the positive control induced a significant ($p \leq 0.05$) increase in MPEs.

   b. **Positive response**: The test material was considered positive for micronuclei induction if a significant increase ($p \leq 0.05$) in MPEs compared to the solvent control was seen, and the response was dose- and/or time-dependent.

5. **Statistical Methods**: The data were evaluated for statistical significance at $p \leq 0.05$ using the Kastenbaum-Bowman tables.

II. REPORTED RESULTS:

A. **Pilot Study**: Animals administered the selected doses of the test material were weighted immediately prior to dosing and on days 1 and 3 postdosing, Clinical signs and mortality were monitored immediately after dosing and daily, thereafter, for 3 days. All animals receiving 5000 mg/kg (5 males and 5 females) died within 24 hours of compound administration. Lethargy was observed in the two males treated with 1000 mg/kg. Doses ≤100 mg/kg were not toxic.

B. **Toxicity Test Trial I**: Based on the results of the pilot study, a second evaluation of toxicity was undertaken with four doses of the test material (800, 1400, 2200 and 3500 mg/kg) administered IP to groups of five male and five female mice. Body weights, mortality and clinical signs were recorded as described in the pilot study. All animals treated with the three highest compound levels succumbed to treatment within one day. Mice receiving the low dose (800 mg/kg) were lethargic for the first day posttreatment but returned to normal by day 3. Since the pattern of mortality did not permit an accurate assessment of the $LD_{50/3}$, the toxicity test was repeated.

C. **Toxicity Test Trial II**: Doses evaluated in the repeat trial were 1000 and 1200 mg/kg administered IP to groups of five male and five female mice per group. Deaths were seen within 1-2 days of dosing in two high dose females; lethargy was reported for both sexes and both treatment groups. Based on the combined data from the three preliminary studies, the estimated $LD_{50/3}$, calculated by probit analysis, was 1213 mg/kg. Accordingly, the high dose selected for the micronucleus assay (≈80% of the $LD_{50/3}$) was 1000 mg/kg.

D. **Micronucleus Assay**:

a. **Analytical determinations**: The analysis of dosing suspensions indicated that the concentration of the test material found in the low, intermediate and high dosing suspensions were 87.2, 93,8 and 94.8%, respectively.

b. **Animal observations**: In the high dose group, one of 20 males and four of 20 females died prior to the scheduled sacrifice and were replaced with animals from the secondary group. No unscheduled deaths occurred in the males or females of the lower treatment groups. Other clinical signs of compound toxicity included lethargy in the mid- and high-dose groups. Piloerection was also seen in one high-dose female. All remaining animals appeared normal throughout the course of study.

c. **Bone marrow analysis**: Summarized results from the micronucleus assay conducted with 1-naphthaleneacetamide technical administered IP to male and female mice are presented in Study Report Table 2, p 16 (see Attachment). As shown, a slight reduction (≈28% of control) in the PCE:NCE ratio was seen in bone marrow cells recovered from the male mice 24 hours after administration of the high dose (1000 mg/kg). Similar reductions were noted in the high-dose females at 48 and 72 hours postexposure. Although reduced PCE:NCE ratios were observed in the males and females receiving the lower test material levels, the response was marginal and not dose dependent. There was, however, no indication of either a clastogenic or aneugenic response in either sex at any dose or harvest time. By contrast, MPEs were significantly ($p \leq 0.05$) increased in male and female mice administered the positive control (CP at 40 mg/kg). From the overall findings, the study authors concluded that 1-naphthaleneacetamide technical was negative in the mouse micronucleus assay.

III. **REVIEWERS' DISCUSSION/CONCLUSIONS**: We agree with the study authors' assessment that 1-naphthaleneacetamide technical was neither clastogenic nor aneugenic in this in vivo assay. The evidence of overt compound toxicity in conjunction with the slightly depressed PCE:NCE ratios seen in the high dose group (1000 mg/kg) indicates that an appropriate range of test material concentrations was evaluated. Additionally, the sensitivity of the test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant results obtained with the positive control (40 mg/kg CP).

We conclude, therefore, that the study provided acceptable evidence that 1-naphthaleneacetamide technical is not genotoxic in this whole animal test system.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

IV. STUDY DEFICIENCIES: None

Attachment[a)]
SUMMARY OF BONE MICRONUCLEUS STUDY WITH TECHNICAL 1-NAPHTHYLANEACETAMIDE
IN ICR MICE

TREATMENT	SEX	TIME (HR)	No. OF MICE	PCE/TOTAL ERYTHROCYTES	MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES	
					Number/1000 PCE's (Mean ± S.D.	Number/PCE's Scored[1]
1% CMC 20 ml/kg	M	24	5	0.53	0.0 ± 0.00	0/5000
		48	5	0.50	0.0 ± 0.00	0/5000
		72	5	0.51	0.0 ± 0.00	0/5000
	F	24	5	0.53	0.2 ± 0.45	1/5000
		48	5	0.61	0.6 ± 0.89	3/5000
		72	5	0.70	0.2 ± 0.45	1/5000
Technical 1-Naphthaleneacetamide 250 mg/kg	M	24	5	0.44	0.2 ± 0.45	1/5000
		48	5	0.48	0.3 ± 0.50	1/5000
		72	5	0.58	0.6 ± 0.55	3/5000
	F	24	5	0.51	0.0 ± 0.00	0/5000
		48	5	0.50	0.2 ± 0.45	1/5000
		72	5	0.73	0.4 ± 0.89	2/5000
500 mg/kg	M	24	5	0.45	0.4 ± 0.55	2/5000
		48	5	0.49	0.6 ± 0.89	3/5000
		72	5	0.67	0.6 ± 0.55	3/5000
	F	24	5	0.46	0.0 ± 0.00	0/5000
		48	5	0.52	1.2 ± 0.45	6/5000
		72	5	0.65	0.6 ± 0.89	3/5000
1000 mg/kg	M	24	5	0.38	0.0 ± 0.00	0/5000
		48	5	0.43	1.2 ± 0.45	6/5000
		72	5	0.57	1.2 ± 1.10	6/5000
	F	24	5	0.49	0.0 ± 0.00	0/5000
		48	5	0.44	0.0 ± 0.00	0/5000
		72	5	0.50	0.4 ± 0.55	2/5000
CP 40 mg/kg	M	24	5	0.50	7.6 ± 2.79	38/5000*
	F	24	5	0.52	9.2 ± 4.92	46/5000*

[1*] : $p \leq 0.05$ (Kastenbaum-Bowman Tables)
[a)] : Table Reproduced from Study Report MRID 43581005, Table 2, page 16.

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch, Health Effects Division (7509C)
EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D.
RRB4, Health Effects Division (7509C)

Signature: Nancy E. McCarroll
Date 08/06/03
Signature: A. Khasawinah
Date Aug. 7, 2003

TXR # 0051958

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation assay in L5178Y/TK$^{+/-}$ Mouse Lymphoma cells; OPPTS 870.5300 [§84-2]; OECD 476.

DP BARCODE: D214872 **SUBMISSION NO.**: S484982

PC CODE: 056001 TOX. CHEM. NO.: 588

TEST MATERIAL (PURITY): 1-Naphthaleneacetamide Technical (98.7%)

SYNONYM(S): Rootone

CITATION: San, R.H.C. and Clark, J.J. 1995. L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU54.702001; 41 pages. Study Completion Date: February 21 1995. MRID 43580202. Unpublished.

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In an in vitro mammalian cell forward gene mutation study (MRID No: 43580202), cultured L5178Y mouse lymphoma cells were exposed to doses of 1-naphthaleneacetamide technical (Lot # I940415; 98.7%) ranging from 10-2000 µg/mL +/-S9 (initial trial) and nonactivated doses of 100-2000 µg/mL or S9-activated levels of 10-250 µg/mL (confirmatory trial). The S9 homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity (i.e, ≤12% total viability) was seen at ≥1250 µg/mL -S9 and ≥150 µg/mL +S9. The positive controls induced the expected response in the target cells in both trials. There was no evidence of a mutagenic effect in the absence of exogenous metabolic activation. However, dose-related increases in the mutation frequency (MF) were seen at 100 and 250 µg/mL +S9 (MFs of 183 and 292x10^{-6} vs. the solvent control MF of 42x10^{-6} -- initial trial). These treatment group values represent MFs that were increased ≈4.4 and 7.0 fold, respectively. At the high dose, however, total growth was only 3%. In the S9-activated confirmatory trial, MFs of 63, 111, 107, 137 and 184x10^{-6} were calculated at 25, 50, 75, 100 and 150 µg/mL, respectively, vs. a

background MF of $33x10^{-6}$. Fold increases ranged from 1.9 at 25 µg/mL (total growth =83%) to 5.6 at 150 µg/mL (total growth = 9%). These findings provide convincing evidence of mutagenesis. It is, however, not clear if the test material is also a clastogen. The induction of small colony mutants, which is thought to represent genetic damage not only at the TK locus but also at multiple linked loci on chromosome 11b, was only seen in the initial trial. We note that 1-naphthaleneacetamide technical was negative in the mouse micronucleus assay (see MRID No. 43581005). These findings are in agreement with the earlier results of a positive mouse lymphoma assay with the ethyl ester of 1-naphthaleneacetic acid (MRID 43580201). However, the issue as to whether the test substance has intrinsic clastogenic activity can only be resolved by performance of an in vitro cytogenetic assay.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 1-Naphthaleneacetamide technical

Description: White powder
Lot/batch number: I 940415
Purity: 98.7%
Receipt date: June 10, 1994
Stability: Expiration date: May 1, 1998
CAS number: 86-86-2
Structure: Not provided
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature, protected from light and moisture. Dosing solutions were analyzed for actual concentration. The report did not indicate whether dosing solutions were adjusted to 100% active ingredient.

2. **Control Materials**:

Negative: None

Solvent/final concentration: DMSO at 1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield final concentrations of 0.25 and 0.5 µL/mL.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) was prepared in DMSO to yield final concentrations of 2.5 and 5.0 µg/mL

3. **Activation**: S9 derived from adult male Sprague-Dawley

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	noninduced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

The S9 liver homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to convert 2-aminoanthracene and DMBA to mutagenic forms in Salmonella typhimurium TA100.

S9 mix composition:

Component	Concentration/mL
NADP	6.0 mg
Isocitric acid	11.25 mg
S9 homogenate	0.25 mL
F_0P	0.75 mL

4. **Test Cells**: Mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		list any others

Media: Fischer's medium supplemented with 0.1% pluronics (F_0P), 10% horse serum, and 4 mM L-glutamine ($F_{10}P$)

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?		Yes	X	No
Periodically checked for karyotype stability?		Yes	X	No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5. **Locus Examined:** X **Thymidine kinase (TK)** **Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)** **Na^+/K^+ ATPase**

Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
3 µg/mL	X	trifluorothymidine (TFT)				

6. **Test Compound Concentrations Used**:

(a) Cytotoxicity assay:

Nonactivated conditions: 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 and 2000 µg/mL
Activated conditions: 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 and 2000 µg/mL

(b) Mutation assay:

(1) Initial Assay:

Nonactivated conditions: Doses of 10-2000 µg/mL were assayed; cultures treated with 100, 250, 500, 750 or 1000 µg/mL were selected for cloning.

S9-Activated conditions: Doses of 10-2000 µg/mL were assayed; cultures treated with 10, 100, or 250 µg/mL were selected for cloning.

(2) Confirmatory Assay:

Nonactivated conditions: Doses ranging from 100-2000 µg/mL were assayed; cultures treated with 500, 750, 1000, 1250 or 1500 µg/mL were selected for cloning.

S9-Activated conditions: Doses of 10-250 µg/mL were assayed; cultures treated with 25, 50, 75, 100, or 150 µg/mL were selected for cloning.

Note: The above selected doses were evaluated in a repeat confirmatory trial. The initial confirmatory trial was aborted owing to lower than expected background mutation frequencies for the nonactivated solvent control cultures and excessive cytotoxicity with S9-activated test material doses of 10-750 µg/mL.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells exposed to the test compound, solvent or positive controls for: 4 hours (nonactivated) 4 hours (activated)

b. After washing, cells cultured for 2 days (expression period) before cell selection

c. After expression, $1x10^4$ cells/plate (3 plates) were cultured for 10 to 12 days in selection medium to determine numbers of mutants and 200 cells/plate (3 plates) were cultured for 10 to 12 days without selection medium to determine cloning efficiency (CE).

d. Colony Sizing Performed Yes If performed list sizing range: 0.2-1.1 mm

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

3. Evaluation Criteria:

a. **Assay validity:** For the assay to be considered valid, the following criteria must be satisfied: (1) CE of the solvent control must exceed 50%; (2) the mutation frequency (MF) of the solvent control was between 20 to 100 mutant colonies/10^6 survivors; and

(3) the MF of the positive controls was ≥2-fold higher than the corresponding solvent control value.

b. **Positive response**: The test material was considered positive if it induced a dose-related increase in the MF that exceeded 2 times the MF of the solvent control at one or more doses with ≥10% total survival.

II. REPORTED RESULTS:

A. **Preliminary Cytotoxicity Assay**: The test material was soluble at all assayed doses (0.5-2000 µg/mL) with or without S9 activation. No appreciable difference between the osmotic pressure of culture medium containing the solvent (DMSO) and the highest test dose was noted. In the nonactivated phase of testing, relative suspension growth (RSG) was 8% at 2000 µg/mL. For the remaining nonactivated levels, RSG was generally dose dependent, ranging from 30% at 1000 µg/mL to ≥100% at the majority of levels ≤100 µg/mL. In the presence of S9 activation, no cells were recovered at the highest concentration and 10% relative cell recovery was reported at 1000 µg/mL. A steep cytotoxicity curve was seen under S9-activated conditions between 500 and 100 µg/mL (22% survival at 500 µg/ml vs. 94% survival at 100 µg/ml). Based on these findings, doses of 10 to 2000 µg/mL +/-S9 were selected for testing in the initial mutation assay.

B. **Mutation Assays**:

Nonactivated conditions: In the initial nonactivated phase of testing, no cells survived treatment with 2000 µg/mL. The RSG of cells postexposure to 1500 µg/mL was reduced to 7%; these cultures were not cloned for mutant selection. RSG was dose dependent for the remaining levels, ranging from 9-23% at 1000 µg/mL to 91% at 100 µg/mL; cultures treated with lower concentrations were not selected for cloning. Additionally, one of the duplicate cultures in the 1000-µg/mL group was lost due to unspecified reasons. As the representative data presented in Table 1 indicate, the nonactivated test material did not induce a mutagenic effect. Data from the confirmatory trial were in good agreement with the initial findings and indicated that 1-naphthalene-acetamide technical was severely cytotoxic at doses ≥1250 µg/mL but not mutagenic. By contrast, the nonactivated positive control (0.25 and 0.50 µl/mL EMS) induced a powerful, concentration-dependent increase in the MF.

S9-activated conditions: Representative results from the S9-activated mutation assays with 1-naphthaleneacetamide technical are presented in Table 2. In the initial trial, no cells survived treatment with levels ≥500 µg/mL; RSG for lower doses was concentration dependent (ranging from 10% at 250 µg/mL to 97% at 10 µg/mL). Accordingly, cells exposed to 10, 100 and 250 µg/mL were cloned. A dose-related

increase in mutant colonies and the MF was seen at 100 and 250 µg/mL; at these levels, average MFs were 183 and $292x10^{-6}$, respectively, compared to an average MF of $42x10^{-6}$ for the DMSO-cultures. Treatment group values correspond to MFs that were increased ≈4.4- and 7.0-fold, respectively. However, total growth (TG) was severely reduced (3% of control) at 250 µg/mL. Based on these findings, a narrower range of test levels (25-200 µg/mL) was processed in the confirmatory trial. The evidence of cytotoxicity and mutagenicity was confirmed in the repeat trial. As shown in Table 2, marked cytotoxicity was achieved at ≥150 µg/mL and mutagenesis was demonstrated at all cloned concentrations. The dose-related increases in the MFs ranged from 5.6-fold over background at 150 µg/mL to 1.9-fold at 25 µg/mL. Although TG at 150 µg/mL was only 9%, the 4.2-fold increased MF at 100 µg/mL ($137x10^{-6}$ vs. $33x10^{-6}$) was accompanied by a TG of 28%. The analysis of colony size distribution showed an increase in the frequency of mutant colonies of all sizes in the initial trial and in the frequency of medium to large colonies in the confirmatory trial compared to the solvent control.

C. **Analytical Determinations**: The results from dosing solution analysis indicated that actual concentrations were within ± 10% of the intended levels.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Based on the overall data, the study authors concluded that 1-naphthalene-acetamide technical was found to be positive in the presence of S9 activation.

B. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the mutation assays were properly conducted, and we agree with the study authors' conclusions that S9-activated 1-naphthaleneacetamide technical was mutagenic in this mouse lymphoma cell forward mutation assay. It is not clear, however, whether the test material also possesses clastogenic properties. Mutant colonies of all sizes including small colony mutants, which are thought to represent multiple locus mutations associated with chromosome 11b alterations[1], were induced in the initial trial. We note that 1-naphthaleneacetamide technical was negative in the mouse micronucleus assay (see MRID No. 43581005). These findings are in agreement with the earlier results of a positive mouse lymphoma assay with the ethyl ester of 1-naphthaleneacetic acid (MRID 43580201). The issue as to whether the test substance has intrinsic clastogenic activity can only be resolved by performance of an in vitro cytogenetic assay.

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[1]Hozier, J., Sawyer, J., Clive, D. and Moore, M.M. (1985). Chromosome 11 aberrations in small colony TK $^{+/-}$ mutants early in their clonal history. Mutat Res. 147: 237-242.

C. STUDY DEFICIENCIES: We note that in the Summary Statement of the Study Report (p.7) it is stated that the frequency of small and medium-sized colonies were increased in both S9-activated trials. However, on Study Report p 14, the statement was made that the frequency of medium to large colonies was increased at the highest dose in the confirmatory trial. Our independent evaluation of the colony size distribution data supports the latter statement.

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with 1-Naphthaleneacetamide Technical

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average % Relative Cloning Efficiency[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Solvent Control							
Dimethyl sulfoxide	1%	100[d]	29	188	100	31	--
(Test material)	1%	100[e]	14	137	100	22	--
Dimethyl sulfoxide	1%	100[d]	25	183	100	27	--
(Positive controls)	1%	100[e]	13	170	100	15	--
Positive Control[f]							
Ethylmethane sulfonate	0.25 µL	71[d]	296	133	73	445	16.5
	0.25 µL	67[e]	304	81	48	751	50.1
Test Material							
1-Naphthaleneacetamide	750 µg[g]	62[d]	32	205	109	32	1.0
Technical	1000 µg[h]	16[i]	36	190	101	38	1.2
	1000 µg[g]	33[e]	17	132	96	28	1.0
	1250 µg	13	17	121	88	29	1.3
	1500 µg[h]	11	12	87	64	24	1.1

[a]The means and standard deviations from the counts of triplicate plates per duplicate culture (test material doses and solvent controls) were presented separately; values for individual cultures were combined by our reviewers. Single cultures were used for each level of the positive controls.

[b]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies}}{\text{Average Viable Colonies}} \times 2 \times 10^{-4}$

[c]Fold Increase = $\frac{\text{MF (test group)}}{\text{MF (solvent control)}}$

[d]Results from the initial trial
[e]Results from the confirmatory trial
[f]Two concentrations of the positive control were assayed; data from the lower dose were selected as representative.
[g]Lower doses (100, 250, or 500 µg/mL--initial trial or 500 or 750 µg/mL--confirmatory trial) did not induce a mutagenic response.
[h]Higher levels 1500 µg/mL--initial trial or 2000 µg/mL) were severely cytotoxic and not cloned.
[i]One of the two replicate cultures was lost; the reason was not specified.

Note: Data were extracted from the study report, pp 16, 17, 20 and 21.

TABLE 2. Representative Results of the S9-activated Mouse Lymphoma Forward Mutation Assays with 1-Naphthaleneacetamide Technical

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average % Relative Cloning Efficiency[a]	Average Percent Total Growth[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Solvent Control								
Dimethyl sulfoxide	1%	100[d]	42	201	100	100	42	--
(Test material)	1%	100[e]	21	126	100	100	33	--
Dimethyl sulfoxide	1%	100[d]	40	180	100	100	45	--
(Positive controls)	1%	100[e]	28	139	100	100	39	--
Positive Control[f]								
7,12-Dimethylbenz(a)-	2.5 µg	86[d]	131	137	76	86	191	4.2
anthracene	2.5 µg	67[e]	120	102	73	50	235	6.0
Test Material								
1-Naphthaleneacetamide	10 µg	97[d]	48	173	86	84	56	1.3
Technical	100 µg	47	133	145	72	34	183	4.4
	250 µg[g]	10	91	62	31	3	292	7.0
	25 µg	93[e]	35	112	89	83	63	1.9
	50 µg	77	59	107	85	66	111	3.4
	75 µg	56	60	113	90	51	107	3.2
	100 µg	35	70	103	82	28	137	4.2
	150 µg[g]	15	68	74	59	9	184	5.6

[a]The means and standard deviations from the counts of triplicate plates per duplicate cultures (test material doses and solvent controls) were presented separately; values for individual cultures were combined by our reviewers. Single cultures were used for each level of the positive controls.

[b]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies}}{\text{Average Viable Colonies}} \times 2 \times 10^{-4}$

[c]Fold Increase = $\frac{\text{MF (test group)}}{\text{MF (solvent control)}}$

[d]Results from the initial trial
[e]Results from the confirmatory trial
[f]Two concentrations of the positive control were assayed; data from the lower dose were selected as representative.
[g]Higher doses (≥500 µg/ml--initial trial or 200 µg/ml--confirmatory trial) were severely cytotoxic.
Note: Data were extracted from the study report, pp 18, 19, 22 and 23.

P

DATA EVALUATION RECORD

1-NAPHTHALENEACETAMIDE/056001

STUDY TYPE: METABOLISM AND PHARMACOKINETICS - RAT
[OPPTS: 870.7485 (§85-1)]
MRID 43963301

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-23

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U.S. Dept. of Energy under contract DEAC0500OR22725

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Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/30/03

TXR#: 0051958

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; [OPPTS 870.7485 (§85-1)]; OECD 417.

PC CODE: 056001

DP BARCODE: D228051
SUBMISSION NO.: S508547

TEST MATERIAL (PURITY): 1-Naphthaleneacetamide (99.7%)

SYNONYMS: Not available

CITATION: McCorquodale, G.Y. and M.S. Prout. 1996. The metabolism of [^{14}C]-1-naphthaleneacetamide in the rat following oral administration. Report No. 12346, Iveresk Research International, Tranent, EH33 2NE, Scotland. 20 March 1996. Study No. IRI 154791. Unpublished, 136 pages. MRID 43963301.

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Blvd., Los Angeles, CA 90023.

EXECUTIVE SUMMARY: In a study (MRID 43963301) conducted to examine the metabolism and disposition of 1-naphthaleneacetamide, five male and five female Sprague-Dawley rats were given either a single 1 or 100 mg/kg bw oral dose, or a 14-day repeated dose (1 mg/kg/day). Groups of male and female rats were subjected to the dosing regimens above using [^{14}C] ring labeled -1-naphthaleneacetamide (Batch No. 94-516-38-10; 99.7% radiochemical purity, specific activity 55.5 mCi/mmol), and nonlabeled test article (Batch No. KP 0100487, chemical purity not available). Excretion, tissue distribution, and metabolite profiles were determined.

There were no biologically significant treatment-related effects noted during the course of the study. Overall recovery of administered radioactivity was an excellent 97.2-101%. 1-Naphthaleneacetamide was readily absorbed and excreted within 36 hours following a single 1 mg/kg bw, a 14-day repeat oral dose of 1 mg/kg bw, or a single 100 mg/kg bw oral dose. Following single or multiple oral low doses (1 mg/kg bw) of [C^{14}]-1-naphthaleneacetamide, urinary excretion accounted for 70.8-74.1% of the administered radioactivity suggesting that a multiple exposure regimen did not affect the absorption/excretion processes. Urinary excretion was unaffected following a single 100 mg/kg dose with 66.2-69.5% of the administered radioactivity excreted in urine. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (21.6-26.2%). Excretory patterns did not

exhibit gender-related variability but reflected delayed absorption in the high-dose group. Because tissue burdens were very low at termination, neither 1-naphthaleneacetamide nor its metabolites appear to undergo significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC and HPLC/MS in conjunction with known standards. Urinary metabolism involved amide cleavage followed by glycine conjugation with the glycine conjugate being the major metabolite of the low and repeat doses (13.7-47.3% of the administered radioactivity). The glucuronide conjugate was also a major metabolite at the low doses (4.5-7.0% of administered). For feces, the major metabolite detected was the dihydrodiol of naphthaleneacetamide (3.6-11.3% of administered. Parent compound was detected at low concentrations (0.7-1.9% of administered) only in feces. Extraction efficiencies appeared to be excellent and most components in the matrices examined (urine and feces) were adequately quantified and characterized. The available data, based upon studies using labeled 1-naphthaleneacetamide, affirmed the metabolism pathway proposed by the investigators.

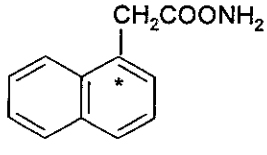
This metabolism study (MRID 43963301) is **Acceptable/Guideline** and satisfies the requirements for a tier 1 Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)] to determine the routes and rate of excretion and to identify excreted metabolites in male and female rats. The study was properly designed, conducted and reported.

COMPLIANCE: Signed GLP, Quality Assurance, and Confidentiality Claim statements were provided in the study reports.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	ring labeled [^{14}C]-1-naphthaleneacetamide
Radiochemical purity	99.7%
Chemical purity	Not available
Specific Activity	55.5 mCi/mmol (11.087 MBq/mg)
Batch #:	94-516-38-10
Non-radiolabelled test material:	1-naphthaleneacetamide
Description:	White powder
Batch #:	KP 0100487
Purity:	Not reported
Contaminants:	Not reported
CAS # of TGAI:	Not reported
Structure:	
*** denotes position of label**	

2. **Vehicle:** The vehicle for labeled and unlabeled 1-naphthaleneacetamide was corn oil. The dosing solutions were prepared by dissolving labeled and unlabeled test materials in acetone and suspending the solution in corn oil. The suspensions were stirred under nitrogen to remove all traces of acetone.

3. **Test animals:**

Species:	Rat (male and female)	
Strain:	Sprague-Dawley	
Age/weight at study initiation:	7-8 weeks; 170-418 g (males), 134-248 g (females)	
Source:	Charles River (UK) Ltd.	
Housing:	Housed up to 3/cage in polypropylene and stainless steel cages during acclimation and in all-glass metabolism cages during experiments. High dose rats were placed in metabowls for predose collection of urine and feces.	
Diet:	SDS Rat and Mouse Maintenance Diet No. 1, Special Diet Services, Essex, UK; ad libitum	
Water:	Tap water; ad libitum	
Environmental conditions:	**Temperature:**	Recorded daily, results not available
	Humidity:	Recorded daily, results not available
	Air changes:	Not reported
	Photoperiod:	Not reported
Acclimation period:	Animals were acclimatized at least 7 days prior to study initiation.	

4. **Preparation of dosing solutions:** The low dose suspension (1 mg/kg bw) was prepared by dissolving appropriate amounts of labeled 1-naphthaleneacetamide in acetone and adding this to a weighed amount of corn oil. The acetone was evaporated under nitrogen with stirring. The low repeat labeled dose (1 mg/kg bw) was prepared in the same manner. In addition an unlabeled suspension was prepared for the 14 consecutive daily doses by dissolving an appropriate amount of unlabeled 1-naphthaleneacetamide in acetone and adding this to a weighed amount of corn oil. The acetone was evaporated under nitrogen with stirring. The high dose suspension (100 mg/kg bw) was prepared by dissolving appropriate amounts of [^{14}C]-labeled and unlabeled 1-naphthaleneacetamide in acetone and adding this to an appropriate weighed portion of corn oil. The acetone was evaporated under nitrogen with stirring. Homogeneity of several aliquots and radioactivity content of the dosing suspensions were determined at the time of dose preparation, just prior to dosing, and during dosing. The stability of the dosing suspensions was determined by measuring the radioactivity over a 14 day period. The radioactive purity was determined by TLC to be 97.9% after 14 days.).

B. STUDY DESIGN AND METHODS:

1. **In life dates** - Start: 11/17/94 End: 11/13/95

2. **Group arrangements:** Animals were assigned to the experimental groups based upon body weights. The groups were established to minimize standard deviation in group mean body weight for each group.

TABLE 1. Experimental design: metabolism and disposition of [^{14}C]-1-naphthaleneacetamide in rats			
Experimental Group	**Dose (mg/kg)**	**Number/ sex**	**Remarks**
1	1 1	5♂ 5♀	Absorption, distribution, excretion, metabolism study; periodic collection of urine, feces. Tissues and carcass at terminal sacrifice (168 hrs.).
2	1 1	5♂ 5♀	14-day non-labeled 1-naphthaleneacetamide followed by single dose of [^{14}C]-1-naphthaleneacetamide on Day 15; periodic collection of urine, feces. Tissues and carcass at terminal sacrifice at 168 hrs. following administration of the radioactive dose.
3	100 100	5♂ 5♀	Absorption, distribution, excretion, metabolism study; periodic collection of urine, feces. Tissues and carcass at terminal sacrifice (168 hrs.).

Information taken from pp. 19-21, MRID 43963301.

3. **Dosing and sample collection:** Animals were dosed by gastric gavage. Doses were drawn into a tared syringe and administered. The administered dose was calculated from the difference in weight of the syringe before and after dosing.

a. **Pharmacokinetic Studies**

Expired air: Expired air was not monitored for radioactivity during these experiments.

Urine: Urine was collected from rats in Groups 1, 2, and 3 at 0-6, 6-12, 12-24, 24-36, 36-48 hours and at 24-hour intervals to termination at 168 hours post-dose. For the first 48 hours, urine was collected over dry ice. Samples were analyzed for total radioactivity immediately or stored frozen at *ca* -20°C.

Feces/cage wash: Feces were collected from rats in Groups 1, 2, and 3 at 12-hour intervals to 48 hours, then at 24-hour intervals to termination. Feces were analyzed for total radioactivity immediately or stored frozen at *ca* -20°C. Cage washings using water were collected at each fecal collection interval and retained at room temperature for subsequent analysis.

Tissue/organs: The following organs/tissues were collected from all rats at terminal sacrifice: bone, brain, perirenal fat, testes/ovaries, heart, liver, kidneys, lungs, whole blood, plasma, spleen, muscle (site not specified), gastrointestinal tract with contents, and carcass. Whole blood samples were held at 4°C until analysis. Tissues and organs were analyzed immediately for total radioactivity or held frozen at *ca* -20°C.

b. **Analytical techniques and metabolite characterization studies:**

Liquid scintillation counting (LSC): LSC was conducted using a Packard 1600TR Liquid Scintillation Analyzer (Canberra Packard Limited) with external standardization. Liquid samples (urine, plasma, and cage wash) were added in duplicate to Quickzint (Zinsser) scintillant and counted directly.

Combustion Analysis: Total radioactivity in feces, tissues/organs, and carcass was determined by combustion analysis using a Model 306 Tri-Carb Automatic Sample Oxidizer (Canberra Packard Limited). Samples were weighed into Combustocones (Packard Instruments Company Limited) in duplicate, combusted, and the resultant $^{14}CO_2$ was collected in Carbosorb and automatically mixed with Permaflour E scintillation fluid. Total

radioactivity in combusted samples was determined using LSC. Combustion efficiency was periodically determined to be >96% using a combustion quality control standard (Spec-Check-^{14}C). Duplicate samples of feces were weighed, homogenized with water using a Waring blender, and combusted. Whole blood and individual ovaries were weighed into combustocones in duplicate and combusted. Other tissue/organ samples were weighed, chopped finely, and duplicate samples combusted. Carcass samples were minced, homogenized, and duplicate samples of the homogenate combusted. Combusted samples were collected in Carbosorb and aliquots added to scintillant for radioassay.

High performance liquid chromatography (HPLC): HPLC was performed with a Hewlett Packard 1050 chromatograph using column/solvent/detection systems that were adequately described in the study report. Aliquots of pooled 0-24 hour urine samples from Group 1, 2, and 3 males and females were directly injected for HPLC analysis without further treatment. Pooled 0-48 hour feces from Group 1, 2, and 3 males and females were extracted with acetonitrile/ammonium formate (7:3 v/v). Aliquots of the extracts were injected for HPLC chromatography.

Thin-layer chromatography (TLC): TLC was performed on Merck silica gel plates to determine the radiochemical purity of the radiolabeled test article, [^{14}C]-1-naphthaleneacetamide using the following solvent systems: hexane:ethyl acetate (10:90, v/v), hexane:ethyl acetate (20:80, v/v), and dichloromethane:methanol (95:5, v/v). Radioactive areas of the developed plates were quantified using an Isomess IM-3016 Radio-TLC analyzer.

HPLC Mass spectrometry (LC-MS): As required, various metabolites were identified by mass spectrometry by comparison to spectra of authentic standards using a VG Biotech Platform mass spectrometer coupled with a HP 1050 high performance liquid chromatograph.

5. **Statistics:** Group means and standard deviations were calculated and presented. Other group statistical comparisons were not performed.

II. RESULTS:

A. **PHARMACOKINETIC STUDIES:** Recovery of radioactivity was an excellent 97.2-101.0%. A general accounting of recovered radioactivity following dosing with [^{14}C]-1-naphthaleneacetamide is summarized in Table 2. Radioactivity in expired air was not monitored during this study.

TABLE 2. Recovery of radioactivity (% of administered dose) from rats at 168 hours following oral administration of [^{14}C]-1-naphthaleneacetamide						
Compartment	Dose group					
	1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
	Males	Females	Males	Females	Males	Females
Tissues/Organs/ Carcass	0.11±0.02	0.17±0.03	0.18±0.11	0.13±0.04	0.23±0.05	0.33± 0.09
Urine	74.1±4.94	70.8±6.17	73.7±3.25	71.0±7.93	66.2±5.11	69.5±8.13
Feces	21.6±5.04	26.2±5.40	21.1±2.21	24.3±7.32	25.4±5.55	21.7±4.36
Cage wash	4.25±3.64	4.81±2.32	2.23±1.26	2.16±0.99	7.37±3.62	7.56±4.02
Total	100±2.50	101±0.99	97.2±0.95	97.6±1.11	99.1±0.20	99.3±1.24

Data obtained from Tables 1, 4, and 7, pp 38, 41, and 44, MRID 43963301.

Absorption/excretion/kinetics:

Absorption: Absorption can be implied from urine, cage wash, carcass, and tissue burden data collected in the absorption/distribution/metabolism/excretion phase of the study. Based upon these data, absorption following a single low dose or single high dose, respectively, was approximately 75.8-78.56% and 73.8-77.4%. Results from the 14-day repetitive dose study (73.2-76.1%) showed an excretion profile similar to that of the single low-dose or single high-dose groups and therefore, similar implied absorption. Adsorption rates appeared similar in both males and females. The excretion time-course suggested that absorption occurred within 12 hours for the single and repeat-low dose groups and between 12 to 24 hours for the high-dose group.

Excretion: Time-course excretion data are summarized in Tables 3 and 4. The data indicated that most of the administered dose is excreted in the urine; 70.8-74.1% for the low dose-group, 66.2-69.5% for the high-dose group, and 71.0- 73.7% for the repeated low-dose group. Greater than 90% (98.0-99.1%) of the urinary excretion of administered radioactivity occurred within 24 hours for the low-dose rats and high dose rats (90.3-92.8%). For high-dose rats, urinary excretion was essentially complete (98.4-98.6%) within 36 hours. Fecal excretion was relatively rapid and essentially complete within 36 hours for low-dose rats (98.6-99.6%) and 48 hours for high dose rats (99.1-99.2%). There were no biologically relevant gender-related quantitative differences in excretion profiles. Urinary excretion time-course was somewhat more prolonged in the high-dose group (peak at 12-24 hours) relative to the low-dose group (~6-12 hours). Time-course for urinary and fecal excretion in the repetitive low-dose group were similar to those of the single low-dose group.

TABLE 3. Time-course of urinary excretion (% of administered dose) in rats orally administered [^{14}C]-1-naphthaleneacetamide

Time (hrs)	Dose group					
	1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
	Males	Females	Males	Females	Males	Females
0-6[a]	47.3	33.9	16.1	12.4	22.7	16.6
6-12	22.5	29.8	47.8	47.6	16.7	15.0
12-24	3.6	5.7	8.1	9.5	20.4	32.9
24-36	0.4	0.7	1.1	1.0	5.5	3.9
36-48	0.2	0.3	0.4	0.2	0.5	0.5
48-72	0.0	0.2	0.1	0.1	0.2	0.2
72-96	0.1	0.0	0.0	0.1	0.1	0.1
96-120	0.0	0.1	0.1	0.0	0.0	0.1
120-144	0.0	0.0	0.0	0.0	0.0	0.1
144-168	0.0	0.1	0.0	0.1	0.1	0.1
Total	74.1	70.8	73.7	71.0	66.2	69.5

Data taken from Tables 1, 4, and 7, pp. 38, 41, and 44, MRID 43963301.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

TABLE 4. Time-course of fecal excretion (% of administered dose) in rats orally administered [^{14}C]-1-naphthaleneacetamide

Time (hrs)	Dose group					
	1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
	Males	Females	Males	Females	Males	Females
0-12[a]	14.5	14.7	5.1	5.6	5.6	5.2
12-24	6.5	9.2	13.4	17.1	15.7	13.8
24-36	0.2	1.9	1.4	0.0	3.1	2.0
36-48	0.3	0.3	1.0	1.5	0.8	0.5
48-72	0.1	0.1	0.1	0.1	0.1	0.1
72-96	0.0	0.0	0.0	0.0	0.0	0.0
96-120	0.0	0.0	0.0	0.0	0.0	0.0
120-144	0.0	0.0	0.1	0.0	0.0	0.0
144-168	0.0	0.0	0.0	0.0	0.1	0.1
Total	21.6	26.2	21.1	24.3	25.4	21.7

Data taken from Table 1, 4, and 7, pp. 38, 41, and 44, MRID 43963301.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

Biliary excretion was not assessed.

Plasma pharmacokinetics: Plasma pharmacokinetics were not evaluated in the study.

B. TISSUE DISTRIBUTION: The distribution of the administered radioactivity tissues, organs and carcass, determined at terminal sacrifice (168 hours), is shown in Table 5. A time-course of tissue distribution was not performed for this study. The highest tissue burdens in low dose animals were found in the kidneys, liver, lungs, renal fat, residual carcass, and whole blood with radioactive residues being generally higher in females than males. Mean tissue burdens at termination accounted for only 0.11% and 0.17% of the administered radioactivity for males and females, respectively. Whole blood accumulated significantly more radioactivity than plasma indicating that the site of accumulation in blood may be the red

blood cells. For repeat dose animals the highest tissue burdens were found in the kidneys, liver, lungs, renal fat, residual carcass and whole blood and, as with low dose animals, radioactive residues in these tissues were generally higher in females than males. For the repeat dose animals, mean tissue burdens at termination accounted for only 0.18% and 0.13% of the administered radioactivity for males and females, respectively. For the high-dose group tissue burdens were considerably higher than in the low and repeat dose groups as would be expected due to a 100-fold increase in dose. Again, the highest tissue burdens were found in the kidneys, liver, lungs, renal fat, residual carcass and whole blood. Tissue burdens were generally higher in females than in males. The highest tissue burdens for the high-dose group were found in the renal fat of females (2.02 µg/g). As with the low and repeat dose groups, high-dose animals accumulated significantly more radioactivity in whole blood than in plasma indicating possible accumulation by red blood cells. Mean tissue burdens at termination accounted for only 0.23% and 0.33% of the administered radioactivity for males and females, respectively.

There was no evidence of significant bioaccumulation in any tissue or organ at 168 hours post-dosing. Regardless of dose or sex, the mean total accumulation of radioactivity in organs, tissues and carcass was <1% of the administered dose at termination (0.11-0.33%).

TABLE 5. Total radioactive residue (µg/g) in organs and tissues in rats 168 hrs following oral administration of [14C]-1-naphthaleneacetamide

Organ/Tissue	Dose Group					
	1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
	Males	Females	Males	Females	Males	Females
GI Tract	0.0002±0.0001	0.0003±0.0001	0.0006±0.0003	0.0003±0.0001	0.06±0.01	0.17±0.05
Carcass	0.0009±0.0001	0.0016±0.0003	0.0022±0.013	0.0012±0.0004	0.20±0.04	0.31±0.10
Bone	0.0002±0.0001	0.0004±0.0002	0.0003±0.0001	0.0004±0.0002	0.04±0.03	0.02±0.01
Brain	0.0001±0.0001	0.0001±0.0001	0.0000±0.0000	0.0001±0.0001	0.02±0.01	0.01±0.01
Renal Fat	0.0007±0.0005	0.0012±0.0006	0.0007±0.0002	0.0007±0.0002	0.32±0.31	2.02±1.20
Heart	0.0003±0.0003	0.0008±0.0006	0.0002±0.0000	0.0011±0.0008	0.10±0.08	0.08±0.10
Kidneys	0.0023±0.005	0.0038±0.0014	0.0034±0.0005	0.0067±0.0026	0.33±0.18	0.32±0.13
Liver	0.0025±0.0015	0.0038±0.0016	0.0033±0.0007	0.0048±0.0025	0.47±0.21	0.39±0.22
Lungs	0.0012±0.0007	0.0027±0.0016	0.0009±0.0001	0.0033±0.0021	0.20±0.13	0.16±0.12
Muscle	0.0002±0.0001	0.0003±0.0001	0.0002±0.0001	0.0003±0.0002	0.04±0.02	0.07±0.03
Ovaries	NA	0.0007±0.0003	NA	0.0008±0.0003	NA	0.61±0.38
Plasma	0.0002±0.0000	0.0002±0.0000	0.0003±0.0001	0.0002±0.0001	0.01±0.01	0.02±0.00
Spleen	0.0005±0.0006	0.0016±0.0008	0.0005±0.0001	0.0030±0.0021	0.17±0.13	0.16±0.12
Testes	0.0001±0.0000	NA	0.0001±0.0001	NA	0.04±0.01	NA
Whole Blood	0.0031±0.0030	0.0070±0.0039	0.0016±0.0004	0.0083±0.0073	0.80±0.62	0.59±0.63

Data obtained from Tables 2, 5, and 8, pp. 39, 42, and 45, MRID 43963301.

C. METABOLITE CHARACTERIZATION STUDIES:

Based upon HPLC and HPLC/MS analyses, most of the metabolite fractions were identified as follows:

NAADHD	Polar metabolite (tentatively identified as dihydrodiol)
NAA	Naphthaleneacetic acid
NAA-Gluc	Naphthaleneacetic acid glucuronide conjugate
NAA-Glyc	Naphthaleneacetic acid glycine conjugate
HO-NAA	Hydroxy-naphthaleneacetic acid
NA-amide	1-Naphthaleneacetamide

The relative proportions of metabolites identified in 0-24 hour urine and 0-48 hour feces extracts are given in Tables 6 and 7, respectively.

Urine: The urinary metabolite profile for rats given [^{14}C]-1-naphthaleneacetamide included seven metabolite fractions, three of which were isomers of HO-NAA (Table 6.). All were recovered at >1% of the administered dose. The major metabolites detected were the glucuronide and glycine conjugates of naphthaleneacetic acid (4.5-18.1% and 13.7-47.3% of the administered dose, respectively) with the glycine conjugate being identified as the predominate metabolite, especially in low-dose males. Other metabolites detected in urine were HO-NAA isomers (8.8-19.8 % of administered), NAADHD (1.8-9.6% of administered), and NAA (2.2-3.1% of administered). At the low single dose and repeat dose, females tended to exhibit less overall metabolism than males and less of each major metabolite was present. Generally the opposite appeared to be the case at the high dose. However no qualitative differences were noted for males or females in any dose group. Parent compound was not detected in urine.

TABLE 6. Metabolite profile in urine of rats following oral dosing with [^{14}C]-1-naphthaleneacetamide expressed as % of urinary radioactivity and (% of administered dose)

Metabolite	RT[a]	Dose group					
		1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
		Males	Females	Males	Females	Males	Females
NAADHD	3.6	6.6 (4.9)	13.6 (9.6)	2.4 (1.8)	9.7 (6.9)	7.6 (5.0)	5.9 (4.1)
HO-NAA[b]	4.3	5.9 (4.4)	10.0 (7.1)	4.8 (3.5)	8.3 (5.9)	7.6 (5.0)	4.7 (3.3)
HO-NAA	5.9	9.4 (7.0)	15.2 (10.8)	4.9 (3.6)	12.0 (8.5)	7.4 (4.9)	8.3 (5.8)
NAA-Gluc	8.4	9.5 (7.9)	8.7 (6.2)	8.1 (6.0)	6.3 (4.5)	19.4 (12.8)	26.0 (18.1)
NAA-Glyc	9.0	45.8 (33.9)	19.3 (13.7)	64.2 (47.3)	32.9 (23.4)	20.7 (13.7)	31.1 (21.6)
HO-NAA	10.6	1.7 (1.3)	2.7 (1.9)	2.3 (1.7)	4.3 (3.1)	6.4 (4.2)	3.7 (2.6)
NAA	12.5	4.0 (3.0)	4.1 (2.9)	3.2 (2.4)	3.1 (2.2)	3.3 (2.2)	4.4 (3.1)
Unaccounted[c]		11.7	19.6	7.4	16.5	18.4	10.9
Identified metabolites as % of recovered		82.9 (61.5)	73.6 (52.2)	89.9 (66.3)	76.6 (54.5)	72.4 (47.8)	84.1 (58.6)
% Dose excreted in urine		74.1	70.8	73.7	71.0	66.2	69.5
Total[d]		83.0	73.8	89.9	76.8	72.2	84.3

Data from Table 10, p. 47, MRID 43963301.
[a]RT = HPLC retention time in minutes
[b]Identity of metabolite based on analogous metabolite of naphthaleneacetic acid, ethyl ester (MRID 43961701)
[c] Unaccounted = Total urinary recovery (% of administered dose) - Total Identified/accounted
[d]Total = Identified metabolites as % of total urinary radioactivity (Total identified/accounted ÷ Total urinary recovery)

Feces: The fecal metabolite profile for rats given [^{14}C]-1-naphthaleneacetamide is given in Table 7. Eight fractions were identified including three fractions identified as isomers of HO-NAA. The major metabolite isolated from feces was NAADHD (3.6-11.3% of administered). Other metabolites identified were NAA-Gluc (1.2-2.7%), NAA-Gly (1.8-3.2%), HO-NAA isomers (1.2-5.5%), and NAA (0.9-3.6% of administered). Parent compound was not the prevalent component extracted from feces (0.7-1.9% of administered) and total fecal metabolites accounted for 21.1-25.4% of the administered dose. Similar to urine metabolite profiles, there were no significant qualitative differences between males and females but marked quantitative differences were observed. Metabolism of 1-naphthaleneacetamide was primarily by amide cleavage.

TABLE 7. Metabolite profile in feces of rats following oral dosing with [^{14}C]-1-naphthaleneacetamide expressed as % of fecal radioactivity and (% of administered dose)

Metabolite	RT[a]	Dose group					
		1 mg/kg bw single dose		1 mg/kg bw repeat dose		100 mg/kg bw single dose	
		Males	Females	Males	Females	Males	Females
NAADHD	3.6	41.0 (8.9)	44.7 (11.3)	17.2 (3.6)	18.5 (4.5)	30.1 (7.6)	27.0 (5.9)
HO-NAA[b]	4.3	4.8 (1.0)	3.7 (0.9)	– (–)	6.3 (1.5)	4.7 (1.2)	– (–)
HO-NAA	5.9	6.8 (1.5)	4.5 (1.1)	5.8 (1.2)	16.4 (4.0)	3.9 (1.0)	2.6 (0.6)
NAA-Gluc	8.4	9.5 (2.1)	9.4 (2.4)	12.6 (2.7)	9.8 (2.4)	9.0 (2.3)	5.6 (1.2)
NAA-Glyc	9.0	9.7 (2.1)	7.8 (2.0)	12.1 (2.6)	8.1 (2.0)	12.7 (3.2)	8.2 (1.8)
HO-NAA	10.6	– (–)	8.1 (2.0)	– (–)	– (–)	– (–)	2.8 (0.6)
NA-Amide	11.5	4.7 (1.0)	2.7 (0.7)	5.4 (1.1)	2.4 (0.6)	7.4 (1.9)	3.5 (0.8)
NAA	12.5	6.8 (1.5)	3.5 (0.9)	16.9 (3.6)	4.8 (1.2)	6.9 (1.8)	9.7 (2.1)
Unaccounted[c]		6.5	3.3	6.9	7.1	9.6	8.7
Identified metabolites as % of recovered		83.3 (18.1)	84.4 (21.3)	70.0 (14.8)	66.3 (16.2)	74.7 (19.0)	59.4 (13.8)
% Dose excreted in feces		(21.6)	(26.2)	(21.1)	(24.3)	(25.4)	(21.7)
Total[d]		83.8	86.9	67.3	70.8	62.2	59.9

Data from Table 11, p. 48, MRID 43963301
[a]RT = HPLC retention time in minutes
[b]Identity of metabolite based on analogous metabolite of naphthaleneacetic acid, ethyl ester (MRID 43961701)
[c] Unaccounted = Total urinary recovery (% of administered dose) - Total Identified/accounted
[d]Total = Identified metabolites as % of total fecal radioactivity (Total identified/accounted ÷ Total recovered in feces)

Plasma: Metabolite profiles were not obtained for plasma.

Tissues: Metabolite profiles were not obtained for tissues.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Overall recovery of radioactivity among the various experimental groups ranged from 97.2-101%. Following oral administration, 1-naphthaleneacetamide was rapidly absorbed and excretion was nearly complete in 24 hours at the low-dose and 36 hours at the high-dose. At sacrifice (7 days post dose), tissue residues of the administered radioactivity were low (<0.5% of the dose) with the highest concentrations found in the liver, lungs, renal fat, kidneys, whole blood and residual carcass. Urine was the major route of excretion (66.2-74.1% of administered dose) with >90% of the urinary excretion occurring within 24 hours. Feces accounted for 21.5-26.2% of the administered dose. Only a small percentage of the dose (0.6-1.9%) was excreted as parent compound. Excretion data did not indicate biologically important, gender-related quantitative differences in excretion profiles but did suggest delayed absorption at the high dose.

The metabolite profile for urine indicated the glycine conjugate of naphthaleneacetic acid to be the major metabolite of the low and repeat doses (13.7-47.3% of administered). The glucuronide conjugate was also a major metabolite at the low doses (4.5-7.9% of administered) but was present in comparatively higher concentrations for high-dose animals (12.8-18.1% of administered). This dose dependent metabolism was even more apparent in u.v. chromatograms than in the HPLC radiochromatograms from which data in Tables 6 and 7 were derived. Other minor metabolites identified in urine were NAADHD, HO-NAA

isomers, and NAA (1.8-9.6, 8.8-19.8, and 2.2-3.1% of administered, respectively). For feces, the major metabolite detected was NAADHD (3.6-11.3% of administered). Parent compound (NA-amide), not detected in the urine, occurred at low concentrations (0.7-1.9% of administered dose) in feces. The study authors proposed the metabolic scheme depicted in Figure 1 for the metabolism of 1-naphthaleneacetamide. The major pathway involved amide cleavage followed by glycine conjugation at the low dose. At the high dose glucuronide conjugation appeared to play a more important role following amide cleavage.

B. REVIEWER COMMENTS: This study (MRID 43963301) was conducted to examine the metabolism and disposition of 1-naphthaleneacetamide in male and female Sprague-Dawley rats following a single 1 or 100 mg/kg bw oral dose, or a 14-day repeated dose (1 mg/kg/day). Rats were subjected to the dosing regimens above using [^{14}C] ring labeled -1-naphthaleneacetamide (Batch No. 99-516-38-10; 99.7% radiochemical purity; chemical purity not stated) and nonlabeled test article (Batch No. KP 0100487; not characterized). Excretion, tissue distribution, and metabolite profiles were determined.

This was a well-designed and conducted study that adequately described the metabolism and disposition of orally administered 1-naphthaleneacetamide in male and female rats. There were no biologically significant treatment-related effects noted during the course of the study. The data demonstrated that 1-naphthaleneacetamide is readily absorbed and rapidly excreted within 36 hours following a single or repeated oral dose of 1 mg/kg or a single dose at 100 mg/kg bw. Following a single low (1 mg/kg) dose or multiple oral low doses (1 mg/kg) of 1-naphthaleneacetamide, urinary excretion accounted for 70.8-74.1% and 71.0-73.7%, respectively, of the administered radioactivity in males and females, respectively, indicating that a multiple exposure regimen did not affect the absorption/excretion processes. Following a single high-dose (100 mg/kg bw) of 1-naphthaleneacetamide, urinary excretion accounted for 66.2% and 69.5% of the administered radioactivity, respectively. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (21.1-26.2%). There was no apparent gender-related difference in excretion profiles. Although >20% of the administered dose was excreted in feces, it was not considered necessary to perform intravenous administration or perform a bile cannulation study because only 0.6-1.9% of the administered radioactivity was recovered in feces as parent compound. However, without a bile cannulation study, microbial degradation cannot be ruled out as a possible source of the metabolites found in feces. Based upon tissue burden data, 1-naphthaleneacetamide and/or its metabolites do not appear to undergo any significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC and HPLC/MS in conjunction with known standards. The metabolite profile for urine indicated the glycine conjugate of naphthaleneacetic acid to be the major metabolite of the low and repeat doses (13.7-47.3% of administered). The glucuronide conjugate was also a major metabolite at the low doses (4.5-7.9% of administered) but was present in comparatively higher concentrations for high-dose animals (12.8-18.1% of administered). For feces, the major metabolite detected was NAADHD (3.6-11.3% of administered). Parent compound (NA-amide) was not detected in the urine but occurred at low concentrations (0.7-1.9% of administered) in the feces. The reviewer agrees with the proposed metabolic scheme depicted in Figure 1 for the metabolism of 1-naphthaleneacetamide. The major pathway

involved amide cleavage followed by glycine conjugation at the low dose. At the high dose glucuronide conjugation appeared to play a more important role following amide cleavage. Extraction efficiencies appeared to be excellent and most components of the matrices examined (urine and feces) were adequately quantified and characterized.

This metabolism study (MRID 43963301) is **Acceptable/Guideline** and satisfies the requirements for a Tier 1 Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)] to determine the routes and rate of excretion and to identify excreted metabolites in male and female rats. The study was properly designed, conducted and reported.

C. **STUDY DEFICIENCIES:** The labeled and unlabeled test materials were incompletely characterized and the conditions of animal handling were not adequately described. The study outcome was not compromised, however, by these omissions.

Approximately 7-18% of the urinary radioactivity and 3-9% of the fecal radioactivity was not accounted for. It is likely that this radioactivity simply represented minor unidentified components (a common occurrence in metabolism/disposition studies) and, therefore, this omission is not considered a serious flaw in the study.

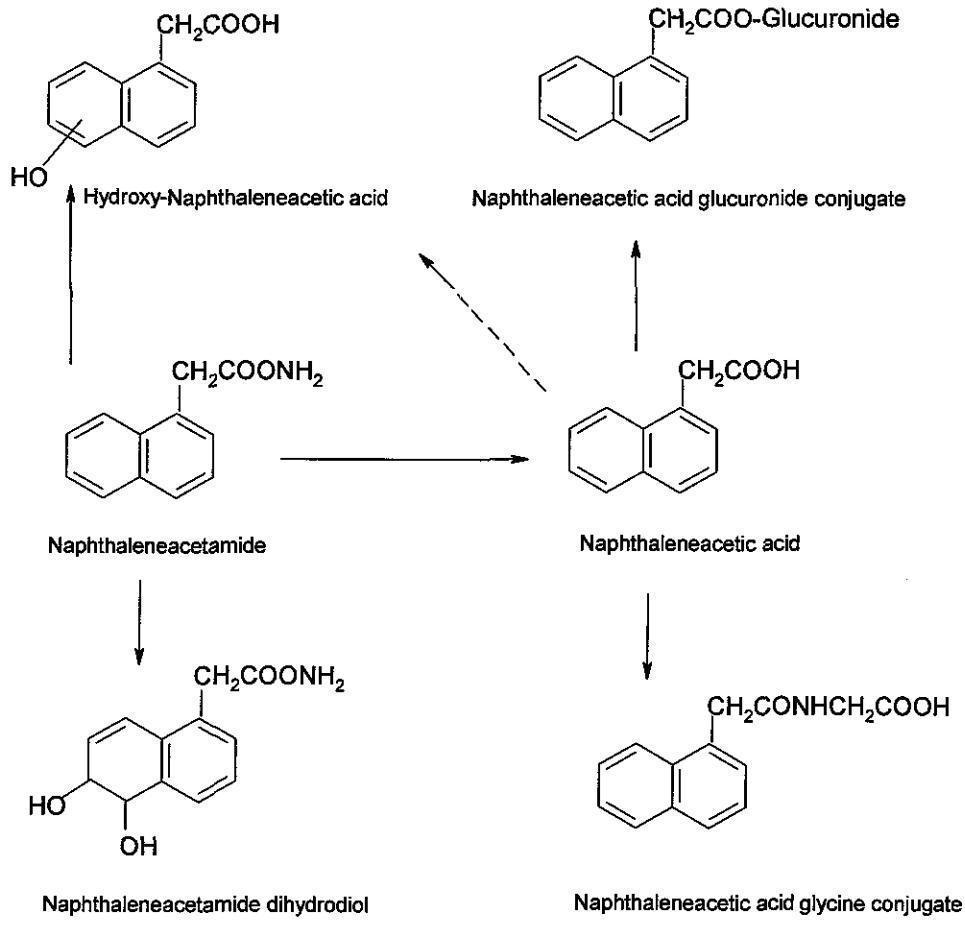


Figure 1. Proposed Metabolic Pathway of 1-Naphthaleneacetamide in the Rat

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