A
1

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

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

TXR No.: 0051960

DATE: January 29, 2004

SUBJECT: **Ethyl 1-Naphthaleneactate**:
PC Code 056008,
Reregistration Case #: 0379

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

A. Khasawinah

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

Susan V. Hummel

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

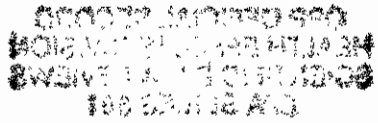
TASK ID: DP Code D210701, D213981, D214872, D223222D229632, D293238

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.

104



1. Acute Oral Toxicity - Rat

In an acute oral toxicity study (MRID 00108831), groups of rats (5/sex) were given NAA ethyl ester (Lot # IA3706; purity not reported, clear liquid) in 0.25% methylcellulose by single dose gavage at the following doses of 1750, 2250, 2500, 2750, 3000, 3500 or 4000 mg/kg bw. Dosage volume was 5 ml/rat.

The oral LD_{50} (95% C.I.) for males and females combined= 2710 mg/kg (2557-2873 mg/kg)

NAA Ethyl Ester is classified as **TOXICITY CATEGORY III**. This acute oral toxicity study in the rat was originally classified core-minimum (HED 005404) but a later review in 1993 (HED 010671) considered it supplementary due to lack of the test substance purity and the use of excessive dosing volumes (5 ml/rat weighing 250 g) exceeding the guideline requirements of 1 ml/100 g bw. Therefore the study is classified **Unacceptable/Guideline** and it does not satisfy the OPPTS 870.1100 [§81-1]; OECD 401 requirement for Acute Oral Toxicity. A more recent **Acceptable/Guideline** study (MRID 43494101) satisfies this requirement.

2. Acute Oral toxicity Study -Rats

In an acute oral toxicity study (MRID 43494101), groups of fasted, young adult Sprague-Dawley rats (5/sex) were given a single oral dose of technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) at doses of 500, 1500, 2250, or 3000 mg/kg bw by gavage (0.455ml/kg at the 500 mg/kg dose to 2.73 ml/kg at the 3000 mg/kg dose) and observed for 14 days.

Oral LD_{50}

Males 2186 mg/kg (95% C.L. 1907-2506 mg/kg),
Females is 2400 mg/kg (95% C.L. 2182-2639 mg/kg), and
Combined is 2300 mg/kg (95% C.L. 2129-2486 mg/kg).

Technical 1-naphthaleneacetic acid, ethyl ester is in EPA **Oral Toxicity Category III**. This acute oral toxicity study is classified as **Acceptable/Guideline**.

3. Acute Dermal Toxicity - Rabbit

In an acute dermal toxicity study (MRID 00108830), five male and five female New Zealand White rabbits received 2 g/kg of 1- naphthaleneacetic acid ethyl ester (Lot # IA3706; purity not reported, clear liquid) on the abraded skin under occlusive wrap for 24 hour exposure. Slight erythema and skin scaling were the only clinical signs observed. Terminal necropsy revealed fluid in the abdominal cavity in two rabbits and a discolored spleen.

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

NAA Ethyl Ester is classified as **CATEGORY III for dermal toxicity**. This acute dermal toxicity study in the rabbit was originally classified core-minimum (HED 005404) but a later review in 1993 (HED 010671) considered it supplementary due to lack of purity information on the test material and the use of abraded skin. 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 it satisfies the OPPTS 870.1200 [§81-2]; OECD 402 requirement for Acute Dermal Toxicity.

4. Acute Dermal Toxicity - Rabbit

In an acute dermal toxicity study (MRID 43494102), five male and five female young adult New Zealand White rabbits were dermally exposed to 2020 mg/kg bw technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot). 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.

Dermal LD_{50}

Males > 2020 mg/kg bw
Females > 2020 mg/kg bw
Combined > 2020 mg/kg bw

Technical 1-naphthaleneacetic acid, ethyl ester 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 43494103), groups of young adult HSD:SD rats (5/sex/group) were exposed by whole body inhalation to 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) for 4 hours at an analytical concentration of 2.13 mg/L. The animals then were observed for 14 days.

Inhalation LC_{50}

Males > 2.13 mg/L
Females > 2.13 mg/L
Combined > 2.13 mg/L

Technical 1-naphthaleneacetic acid, ethyl ester is classified in EPA **inhalationToxicity Category IV.** This acute inhalation study is classified as **Acceptable/Guideline**.

6. Acute Eye Irritation Study - Rabbit

In a primary eye irritation study (MRID 00103052 & 00103217), NAA Ethyl ester (Lot # IA3706; purity not reported, clear liquid) was found to be not an eye irritant in New Zealand White rabbits and was classified as **Category IV** for eye irritation. This study was considered

Acceptable (HED 005404), but a later HED review in 1993 (HED 010671) considered it **Unacceptable** due to lack of purity information on the test material. Therefore, the study is considered **Unacceptable/Guideline**.

7. Acute Eye Irritation - Rabbit

In a primary eye irritation study (MRID 43494104), 0.1 mL by volume of technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) 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 were not noted on any rabbit during the study. One male had positive conjunctival irritation (redness) one hour after test material instillation with resolution by 24 hours. The highest maximum mean total score was 6.0, recorded one hour after test material instillation.

In this study, Technical 1-naphthaleneacetic acid, ethyl ester was minimally irritating to the eye based on the highest maximum mean total score (6.0) recorded one hour after test material instillation. The test material is in EPA Toxicity Category IV. This study is classified as **Acceptable/Guideline**.

8. Acute Dermal Irritation - Rabbit

In a primary dermal irritation study (MRID 00103053 & 00103218), six New Zealand White rabbits received 0.5 ml of NAA ethyl ester (Lot # not provided; 100% purity) at two abraded and two intact skin sites per animal under wrap for 24 hour exposure. Observations were made at 24 and 72 hours after treatment. NAA Ethyl Ester did not produce irritation at 24 or 72 hours. NAA Ethyl Ester is considered non-irritating to the rabbit skin and is classified in **Category IV** for dermal irritation.

A number of deficiencies were identified (HED 010671). These included the use of abraded skin instead of un-abraded skin as the guidelines require, and the dosing exposure should be 4 hours instead of 24 hours. Since the test material was not a skin irritant under these severe test conditions, the current reviewer considers this study **Acceptable/Guideline** and it satisfies the guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.

9. Skin Sensitization - guinea pigs

In a dermal sensitization study (MRID 43494105) with 5% v/v technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) 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 after challenge.

In this study, Technical 1-naphthaleneacetic acid, ethyl ester 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**.

10. 90-Day Oral Toxicity Study - Rats

In a subchronic oral toxicity study (MRID 43896002), 1-naphthaleneacetic acid, ethyl ester (Lot # AM 315002; 100% ai) was administered, in the diet, to CRL:CD BR rats (10/sex/dose) at dose levels of 400, 2000 or 8000 ppm for 13 weeks. The actual average doses at the end of the study were 19-25 mg/kg/day for the 400 ppm group, 92-123 mg/kg/day for the 2000 ppm group, and 388 - 519 mg/kg/day for the 8000 ppm group, for males and females, respectively.

No rats died during the study. No differences in clinical signs, ophthalmology, macroscopic or microscopic pathology were observed between any of the treatment and control groups. Decreased urine protein in the 2000 and 8000 ppm males was not observed in the corresponding females.

The **LOAEL** for this study is 8000 ppm (594 mg/kg/day) for male and female rats, based on lower body weight, suppressed body weight gain, and reduced food consumption as compared to the controls. Absolute and/or relative liver and kidney weights for both sexes were seen at this dose but were not accompanied by any macroscopic or microscopic changes, however, males and females at this dose also exhibited increased total bilirubin (19-21% higher) in conjunction with reduced red blood cell counts, hemoglobin, and hematocrits. The **NOAEL** is 2000 ppm (144 mg/kg/day) for both sexes. This 90-day oral toxicity study in the rat is **Acceptable/Guideline**.

11. 90-Day Oral Toxicity Study - Dogs

In a subchronic oral toxicity study (MRID 43914901), 1-naphthaleneacetic acid, ethyl ester (Lot/Batch # AM 315002; 97.75% ai) was fed (gelatin capsules) to beagle dogs (4/sex/dose) at dose levels of 0, 40, 125, or 400 mg/kg/day for 13 weeks.

All dogs survived the treatment. No differences were observed in clinical blood chemistry, ophthalmology, urine volume or chemistry, organ weights, or macroscopic or microscopic organ morphology between dogs in the treated and the control groups. No neoplastic tissue was observed. The **LOAEL** for this study is 400 mg/kg/day, based on soft/liquid feces and the depressed body weight gains of male and female dogs at this treatment level. Additionally some blood parameters (RBC, hemoglobin, hematocrit and mean platelet volume) were all depressed in the male dogs at this level. The **NOAEL** was 125 mg/kg/day. This 90-day oral toxicity study in the dog is **Acceptable/Guideline.**

12. 21-Day Dermal Toxicity Study - Rat

In a repeated dose dermal toxicity study (MRID 43581002), 1-naphthaleneacetic acid, ethyl ester (Lot # 315002; 97.75% 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.

For all treatment groups, there were no clinical signs of toxicity, and body weights, body weight gains, and food consumption were similar to the controls. There were no differences in hematology parameters, clinical blood chemistry, organ weights, or macroscopic or microscopic organ morphology between rats in the treated and the control groups. No neoplastic tissue was observed. Ophthalmoscopic examinations and urinalysis were not performed during the study. No systemic responses were observed. Therefore, the **LOAEL** for systemic toxicity is >1000 mg/kg/day and the **NOAEL** for systemic toxicity is 1000 mg/kg/day. The **LOAEL** for dermal irritation is 100 mg/kg, based on the presence of treatment-related dermal irritation in the treated skin of rats in the 100, 300, and 1000 mg/kg treatment groups. No **NOAEL** for dermal irritation was established. This 21-day dermal toxicity study in the rat is **Acceptable/Guideline.**

13. Bacterial Gene Mutation - *Salmonella typhimurium*

In a microbial/mammalian microsome plate incorporation mutagenicity study (MRID 43581004), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to five doses of 1-naphthaleneacetic acid, ethyl ester technical (Lot # AM 315002; 97.75%) ranging from 33 to 5000 µg/plate with and without S9 activation. Based on analytical data, actual high doses used in the study were 4195 µg/plate (initial trial) and 3955 µg/plate (confirmatory trial). Two independently performed 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.

Cytotoxicity was observed for all strains at ≥3955 µg/plate -S9 and for the majority of strains at ≈1000 µg/plate -S9. In the presence of S9 activation, cytotoxicity was achieved at 4195 µg/plate. All strains responded in the expected manner to the appropriate positive control. There was, however, no indication that 1-naphthaleneacetic acid, ethyl ester technical induced a mutagenic effect at any dose with or without S9 activation. This study is classified as **Acceptable/Guideline.**

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

In a mouse micronucleus assay (MRID No: 43581003), groups of five male and five female ICR mice received single intraperitoneal injections of 305, 610 or 1220 mg/kg 1-naphthaleneacetic acid, ethyl ester technical (AM 315002; 97.75%). Based on analytical data, the actual doses used in the study were 162.3, 456.3 and 947.9 mg/kg. 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).

Including the secondary group, death occurred in ≈48% (11/20 ♂ and 8/20 ♀) of the high-dose animals. Lethargy was also noted in the high- and mid-dose males and females. 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-naphthaleneacetic acid, ethyl ester technical induced a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. This study is classified as **Acceptable/Guideline**.

15. *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: 43580201), cultured L5178Y mouse lymphoma cells were exposed to doses of 1-naphthaleneacetic acid, ethyl ester technical (Lot # AM 315002; 97.75%) ranging from 10-100 µg/mL -S9 or 50-500 µg/mL +S9 (initial trial) and nonactivated doses of 20-100 µg/mL or S9-activated levels of 60-500 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, ≤25% viability) was seen at 100 µg/mL -S9 and ≤13% total viability was noted at ≥300 µg/mL +S9. The positive controls induced the expected response in the target cells in both trials. There was no evidence that the test material induced a mutagenic effect in the absence of exogenous metabolic activation. However, reproducible increases in the mutation frequency (MF) were seen in the presence of S9 activation. In the initial trial, the MF was increased 3.1-fold at 300 µg/mL (MF =$201x10^{-6}$ vs. $66x10^{-6}$ in the solvent control). The MF was increased ≈2-fold ($128x10^{-6}$ vs. $66x10^{-6}$ in the control) at a comparable dose in the confirmatory trial. Although these findings provide convincing evidence of mutagenesis, the response was confined to a single cytotoxic concentration. 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 further note that 1-naphthaleneacetic acid, ethyl ester technical was negative in the mouse micronucleus assay (see MRID No. 43581003). 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**.

16. Metabolism and Pharmacokinetics - Rat

In a study (MRID 43961701) conducted to examine the metabolism and disposition of 1-naphthaleneacetic acid, ethyl ester, male and female Sprague-Dawley rats were given 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-naphthaleneacetic acid, ethyl ester (Batch No. CSL-94-516-33-25, 99.3% radiochemical purity, specific acttivity 56.2 mCi/mmol), and nonlabeled test article (Batch No. GAB 69-34-02, chemical purity not available). Excretion, tissue distribution, pharmacokinetic parameters, 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 98.6-101.8%. 1-Naphthaleneacetic acid, ethyl ester was readily absorbed and excreted within 36 - 48 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-naphthaleneacetic acid, ethyl ester, urinary excretion accounted for 67.6-85.3% of the administered radioactivity. Urinary excretion was unaffected following a single 100 mg/kg bw dose with 61.8-78% of the administered radioactivity excreted in urine. Excretion via the feces accounted for the remainder of the administered radioactivity excreted by all treatment groups (12.3-35.2%). Excretory patterns did not exhibit gender-related variability for the low dose groups although a minor difference was observed at the high dose. Excretion patterns of the high-dose group reflected delayed absorption. Because tissue burdens were very low at termination, neither 1-naphthaleneacetic acid, ethyl ester nor its metabolites appear to undergo significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC, TLC and most were identified using HPLC, GC/MS, and HPLC/MS in conjunction with known standards. The major pathway of metabolism involved ester cleavage followed by glycine and glucuronide conjugation at the low and low repeat doses. At the high dose, glucuronide conjugation appeared to play a more important role following ester cleavage. Parent compound was detected at low concentrations (0.5-4.7% 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-naphthaleneacetic acid, ethyl ester, affirmed the metabolism pathway proposed by the investigators.

This metabolism study (MRID 43961701) 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.

B

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT
[OPPTS 870.1300 (§81-3) OECD 403]
MRID 43494103

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. 03-22

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: JUL 04 2003

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

Signature: H.T Borges
Date: JUL 04 2003

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

Signature: Robert H. Ross
Date: JUL 04 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L A Wilson
Date: JUL 04 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** 8-27-03
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#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D210701
SUBMISSION NO.: S315600, S511187

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetic acid, ethyl ester (97.75%)

SYNONYMS: Not reported

CITATION: Holbert, M. (1994) Technical 1-Naphthaleneacetic acid, ethyl ester - Acute inhalation toxicity study in rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Laboratory Study No. 1353-94, October 12, 1994. MRID 43494103. Unpublished. 19 pages.

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

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 43494103), groups of young adult HSD:SD rats (5/sex/group) were exposed by whole body inhalation to 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) for 4 hours at an analytical concentration of 2.13 mg/L. The animals then were observed for 14 days.

No animals died during the study. Decreased activity, lacrimation, nasal discharge, piloerection, salivation, and/or ptosis were noted from one male and one female during exposure. The animals recovered from ptosis after removal from the chamber. All animals had piloerection, decreased activity, nasal discharge, and salivation after removal from the chamber and all recovered by day 9. Four females lost weight during the first week of the study and gained weight during the second week. One female lost weight during the second week. The males gained weight throughout the study. Mottled red and slightly swollen lungs were found in two males and two females, possibly related to the administration of the test material.

Inhalation LC_{50}
Males > 2.13 mg/L
Females > 2.13 mg/L
Combined > 2.13 mg/L

Technical 1-naphthaleneacetic acid, ethyl ester is classified in EPA **inhalationToxicity 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-naphthaleneacetic acid, ethyl ester

Description:	Clear, colorless to light brown liquid
Lot/Batch #:	AM 315002
Purity:	97.75% as reported in MRID 43914901 for this lot number.
CAS # of TGAI:	2122-70-5

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

3. **Test animals:**

Species:	Rat	
Strain:	HSD:SD	
Age/weight at dosing:	Young adults; Males: 208-218 g, females: 207-230 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 25, 1994; End: September 8, 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 70-73°F and 90-95%, respectively.

3. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. Rats were exposed to Technical 1-naphthaleneacetic acid, ethyl ester 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 one male and one female could be observed during exposure due to the chamber design and density of the test material. The body weight

was recorded prior to exposure and on days 7 and 14 or at death. Survivors were sacrificed at the end of the study and a necropsy was performed on each animal.

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
7.07	2.13	2.890, 2.462[a]	2.208, 2.153[a]	0/5	0/5	0/10

[a]At 1.25 and 3.25 hours distribution, respectively

4. **Generation of the test atmosphere / chamber description:** The exposure atmosphere during the first half of the exposure was generated by a pressure Spraying System air atomizer (1/4 JSS) which aspirated the test material, and the aerosol was elutriated through a 91 L baffling chamber. The aerosol during the last half of the exposure was generated by pumping the test material into the pressure operated air atomizer and then elutriating the aerosol through the baffling chamber. The aerosol was diluted with dried and filtered air and drawn into the exposure chamber. The average total airflow was 76.4 liters/min and the exposure chamber volume was 500 L. Analytical chemistry was conducted with a Bausch & Lomb Spectronic 2000 Spectrophotometer at 280 nm.

   Test atmosphere concentration: The exposure atmosphere samples were taken from the breathing zone of the animals twice per hour during exposure. The atmosphere 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 and the aerodynamic mass median diameter (MMAD) and geometric standard deviation (GSD) then determined. Results are in Table 1 above. The percent of total particles with sizes of ≤ 3.3 µm are 51-61%.

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

II. RESULTS AND DISCUSSION:

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

   The inhalation LC_{50} for
   males is > 2.13 mg/L,
   females is > 2.13 mg/L,
   combined is > 2.13 mg/L.

B. **CLINICAL OBSERVATIONS:** Decreased activity, lacrimation, nasal discharge, piloerection, salivation, and/or ptosis were noted from one male and one female during exposure. The animals recovered from ptosis after removal from the chamber. All animals had nasal discharge and salivation after removal from the chamber and all recovered by two

hours after removal from the chamber. Salivation subsided by day 2. Piloerection and decreased activity were found in all animals, and all recovered by days 7 (males) or 9 (females).

C. **BODY WEIGHT:** Four females lost weight during the first week of the study and gained weight during the second week, but one female did not regain her original weight and one female regained only her original weight. One female lost weight during the second week. The males gained weight throughout the study.

D. **NECROPSY:** Mottled red and slightly swollen lungs were found in two males and two females.

E. **REVIEWER'S CONCLUSIONS:** 1-Naphthaleneacetic acid, ethyl ester is classified in **inhalation Toxicity Category IV.**

F. **DEFICIENCIES:** None.

C

Supplement to HED Document No. 005404 & 010671 - DER for MRID No. 00108831 - 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 9/11/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D293238

P.C. CODE: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): Ethyl 1-Naphthyl Acetate (purity not reported, clear liquid)

SYNONYMS: NAA Ethyl Ester, Naphthalene Acetic Acid Ethyl Ester

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Oral Toxicity Study in Rats (14 Day): NAA Ethyl Ester: PH 402-UC-003- 82. Pharmakon Research International, Inc. May 3, 1982. MRID 00108831. Unpublished.

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

EXECUTIVE SUMMARY:

In an acute oral toxicity study (MRID 00108831), groups of rats (5/sex) were given NAA ethyl ester (Lot # IA3706; purity not reported, clear liquid) in 0.25% methylcellulose by single dose gavage at the following doses of 1750, 2250, 2500, 2750, 3000, 3500 or 4000 mg/kg bw. Dosage volume was 5 ml/rat. 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= 2710 mg/kg (2557-2873 mg/kg)

Signs of toxicity were piloerection, abnormal gait, abnormal stance, decreased activity and body tone, brown and yellow discoloration of genital and anal area, prostration, body drop, straub tail, diarrhea, ptosis, arched back, tremors, hypersensitivity to touch, chromodacryarrhea, ataxia, vasoconstriction, poor grooming and red discoloration around the oral and nasal cavities. All of

the deaths occurred during the first three days of the study. Necropsy of the animals that died revealed red discoloration around the oral and nasal cavities, pale liver, poor grooming, pulmonary hemorrhages, fluid filled and distended hemorrhagic stomach and intestines. Necropsies at the end of the study revealed distended stomach small hemorrhagic area in the lungs, discolored adrenals and an abscess on a cervical lymph node in several animals.

NAA Ethyl Ester is classified as **TOXICITY CATEGORY III**. This acute oral toxicity study in the rat was originally classified core-minimum (HED 005404) but a later review in 1993 (HED 010671) considered it supplementary due to lack of the test substance purity and the use of excessive dosing volumes (5 ml/rat weighing 250 g) exceeding the guideline requirements of 1 ml/100 g bw. Therefore the study is classified **Unacceptable/Guideline** and it does not satisfy the OPPTS 870.1100 [§81-1]; OECD 401 requirement for Acute Oral Toxicity. A more recent **Acceptable/Guideline** study (MRID 43494101) satisfies this requirement.

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

D

Supplement to HED Document No. 005404 & 010671- DER for MRID No. 00103052 & 00103217 - 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 9/11/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D293238

P.C. CODE: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): Ethyl 1-Naphthyl Acetate (purity not reported, clear liquid)

SYNONYMS: NAA Ethyl Ester, Naphthalene Acetic Acid Ethyl Ester

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Eye Irritation Test in Rabbits: NAA Ethyl Ester: Study NO. PH 421-UC-002-82. May 11, 1982. Pharmakon Research International, Inc. MRID 00103052 & 00103217 . Unpublished.

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

EXECUTIVE SUMMARY:

In a primary eye irritation study (MRID 00103052 & 00103217), 0.1 ml of NAA Ethyl ester (Lot # IA3706; purity not reported, clear liquid) was instilled into the lower right eyelid of each of nine New Zealand White rabbits. The eyes of three rabbits were washed with water immediately after treatment. The other six eyes were unwashed. Observations were made at 24, 48, 72 hours, 4 and 7 days after treatment. Conjunctival redness was noted in one animal only at 24 hours and cleared by 48 hours. No signs of irritation were noted in the other animals.

NAA Ethyl Ester was found in this test to be not an eye irritant and was classified as **Category IV** for eye irritation. This study was considered **Acceptable** (HED 005404), but a later HED review in 1993 (HED 010671) considered it **Unacceptable** due to lack of purity information on the test material. Therefore, the study is considered **Unacceptable/Guideline** and it does not

satisfy the guideline requirement for a primary eye irritation study (OPPTS 870.2400 [§81-4, OECD 405) in the rabbit.

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

E

Supplement to HED Document No. 005404 & 010671- DER for MRID No. 00108830 - 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 9/10/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D293238

P.C. CODE: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): Ethyl 1-Naphthyl Acetate (purity not reported, clear liquid)

SYNONYMS: NAA Ethyl Ester, Naphthalene Acetic Acid Ethyl Ester

CITATION: Mallory, V.; Matthews, R.; Naismith, R.; et al. (1982) Acute Dermal Toxicity Test in Rabbits: NAA Ethyl Ester: PH 422-UC-003-82. Pharmakon Research International, Inc. May 14, 1982. MRID 00108830. Unpublished.

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

EXECUTIVE SUMMARY:

In an acute dermal toxicity study (MRID 00108830), five male and five female New Zealand White rabbits received 2 g/kg of 1- naphthaleneacetic acid ethyl ester (Lot # IA3706; purity not reported, clear liquid) on the abraded skin under occlusive wrap for 24 hour exposure. Observations were made at 2, 4, 24 hour after exposure and daily thereafter for 14 days. Necropsy was performed on all animals. All animals survived the treatment. Slight erythema and skin scaling were the only clinical signs observed. Terminal necropsy revealed fluid in the abdominal cavity in two rabbits and a discolored spleen.

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

NAA Ethyl Ester is classified as **CATEGORY III for dermal toxicity**. This acute dermal toxicity study in the rabbit was originally classified core-minimum (HED 005404) but a later

review in 1993 (HED 010671) considered it supplementary due to lack of purity information on the test material and the use of abraded skin. 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 it 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.

Supplement to HED Document No. 005404 & 010671 - DER for MRID No. 00103053 & 00103218 - Primary Dermal 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 9/10/03
Reregistration Branch 4, Health Effects Division (7509C)

TXR # 0051960

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - (Rabbit); OPPTS 870.2500 [§81-5]: OECD 404.

DP BARCODE: D293238

P.C. CODE: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): Ethyl 1-Naphthyl Acetate (purity 100%)

SYNONYMS: NAA Ethyl Ester, Naphthalene Acetic Acid Ethyl Ester

CITATION: Myers, R.; Mika, E.; Cardella, M.; et al. (1982) Naphthalene Acetic Acid (NAA) Ethyl Ester: Rabbit Skin Irritancy Study: Project Report 45-46. April 22, 1982. Union Carbide Corporation, bushy Run Research Center, Pennsylvania. MRID 00103053 & 00103218. Unpublished.

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

EXECUTIVE SUMMARY:

In a primary dermal irritation study (MRID 00103053 & 00103218), six New Zealand White rabbits received 0.5 ml of NAA ethyl ester (Lot # not provided; 100% purity) at two abraded and two intact skin sites per animal under wrap for 24 hour exposure. Observations were made at 24 and 72 hours after treatment. NAA Ethyl Ester did not produce irritation at 24 or 72 hours. NAA Ethyl Ester is considered non-irritating to the rabbit skin and is classified in **Category IV** for dermal irritation.

A number of deficiencies were identified (HED 010671). These included the use of abraded skin instead of un-abraded skin as the guidelines require, and the dosing exposure should be 4 hours instead of 24 hours. Since the test material was not a skin irritant under these severe test conditions, the current reviewer considers this study **Acceptable/Guideline** and it satisfies the

guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.

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

C

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

STUDY TYPE: ACUTE ORAL TOXICITY - RAT
[OPPTS 870.1100 (§81-1) OECD 401]
MRID 43494101

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. 03-22

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: JUL 04 2003

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

Signature: H.T Borges
Date: JUL 04 2003

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

Signature: Robert H. Ross
Date: JUL 04 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 04 2003

Disclaimer

B. McKay Jr 8/7/03

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** Aug. 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#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D210701
SUBMISSION NO.: S315600, S511187

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetic acid, ethyl ester (97.75%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetic acid, ethyl ester - Acute oral toxicity study in rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Laboratory Study No. 1351-94, September 16, 1994. MRID 43494101. Unpublished. 21 pages.

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

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43494101), groups of fasted, young adult Sprague-Dawley rats (5/sex) were given a single oral dose of technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) at doses of 500, 1500, 2250, or 3000 mg/kg bw by gavage (0.455ml/kg at the 500 mg/kg dose to 2.73 ml/kg at the 3000 mg/kg dose) and observed for 14 days.

Three males and one female in the 2250 mg/kg groups and all animals in the 3000 mg/kg groups died within three days of dosing. The 3000 mg/kg decedents had piloerction and decreased activity on the day of dosing and ataxia, polyuria, body tremors, aggressiveness, and/or lacrimation prior to death. The survivors had piloerection, decreased activity, ptosis, ocular discharge, decreased defecation, and/or nasal discharge. The survivors recovered by day 11 or earlier. One 1500 mg/kg female did not gain weight and one 1500 mg/kg female, three 2250 mg/kg males, and one 2250 mg/kg female lost weight during the second week of the study. The decedents had discoloration of the lungs, liver, fatty tissue, and mesenteric lymph nodes; swollen and undersized lungs; undersized kidneys; gastrointestinal tract distended with gas; and/or discoloration of the contents of the gastrointestinal tract. Some of the survivors had gray pockets in the liver, discolored liver, undersized liver and kidneys, and/or lungs with pinpoint red spots.

Oral LD_{50}
Males 2186 mg/kg (95% C.L. 1907-2506 mg/kg),
Females is 2400 mg/kg (95% C.L. 2182-2639 mg/kg), and
Combined is 2300 mg/kg (95% C.L. 2129-2486 mg/kg).

Technical 1-naphthaleneacetic acid, ethyl ester is in EPA **Oral Toxicity Category III**.

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-naphthaleneacetic acid, ethyl ester

Description:	Clear, colorless to light brown liquid
Lot/Batch #:	AM 315002
Purity:	97.75% as reported in MRID 43914901 for this lot number.
CAS # of TGAI:	2122-70-5

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat	
Strain:	HSD:Sprague-Dawley SD	
Age/weight at dosing:	Young adults; Males: 197-280 g, females: 189-247 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	End
July 6, 1994	July 8 or 9, 1994 3000 mg/kg group
July 13, 1994	July 27, 1994 500 mg/kg group
July 20, 1994	August 3, 1994 1500 mg/kg group
July 27, 1994	August 10, 1994 2250 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 500, 1500, 2250, or 3000 mg/kg technical 1-naphthaleneacetic acid, ethyl ester by gavage, observed at least three times after dosing, and once daily thereafter. 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 treated

Dose (mg/kg bw)	Males	Females	Combined
500	0/5	0/5	0/10
1500	0/5	0/5	0/10
2250	3/5	1/5	4/10
3000	5/5	5/5	10/10

3. **Statistics:** Calculation of the oral LD_{50} was by utilizing probit analysis.

II. RESULTS AND DISCUSSION:

A. **MORTALITY** is given in Table 1. Three males and one female in the 2250 mg/kg groups and all animals in the 3000 mg/kg groups died within three days of dosing.

The oral LD_{50} for
males is 2186 mg/kg (95% C.L. 1907-2506 mg/kg),
females is 2400 mg/kg (95% C.L. 2182-2639 mg/kg), and
combined is 2300 mg/kg (95% C.L. 2129-2486 mg/kg).

B. **CLINICAL OBSERVATIONS:** The 3000 mg/kg decedents had piloerction and decreased activity on the day of dosing and ataxia, polyuria, body tremors, aggressiveness, and/or lacrimation prior to death. The survivors had piloerection, decreased activity, ptosis, ocular discharge, decreased defecation, and/or nasal discharge. The survivors recovered by day 11 or earlier.

C. **BODY WEIGHT:** One 1500 mg/kg female did not gain weight and one 1500 mg/kg female, three 2250 mg/kg males, and one 2250 mg/kg female lost weight during the second week of the study. All other surviving animals gained weight during the study.

D. **NECROPSY:** The decedents had discoloration of the lungs, liver, fatty tissue, and mesenteric lymph nodes; swollen and undersized lungs; undersized kidneys; gastrointestinal tract distended with gas; and/or discoloration of the contents of the gastrointestinal tract. Some of the survivors had gray pockets in the liver, discolored liver, undersized liver and kidneys, and/or lungs with pinpoint red spots.

E. **REVIEWER'S CONCLUSIONS:** Technical 1-naphthaleneacetic acid, ethyl ester is in EPA **Oral Toxicity Category III**.

F. **DEFICIENCIES:** None.

H

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

STUDY TYPE: ACUTE DERMAL TOXICITY - RABBIT
[OPPTS 870.1200 (§81-2) OECD 402]
MRID 43494102

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. 03-22

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: JUL 04 2003

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

Signature: H T Borges
Date: JUL 04 2003

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

Signature: Robert H. Ross
Date: JUL 04 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 04 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#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D210701
SUBMISSION NO.: S315600, S511187

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetic acid, ethyl ester (97.75%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetic acid, ethyl ester - Acute dermal toxicity study in rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Laboratory Study No. 1352-94, September 14, 1994. MRID 43494102. Unpublished. 14 pages.

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

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 43494102), five male and five female young adult New Zealand White rabbits were dermally exposed to 2020 mg/kg bw technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot). 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.

No animals died during the study. Decreased defecation, diarrhea, soft feces, and/or nasal discharge were noted in a few males and females starting on day 1 or later with recovery by day 12 or earlier. Erythema was noted on 5/10 animals with clearance by day 10 and desquamation was noted on five animals. All animals gained weight during the study. One female had the small intestine filled with a green liquid and one female had the large intestine filled with gas at necropsy.

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

Technical 1-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester

Description:	Clear, colorless to light brown liquid
Lot/Batch #:	AM 315002
Purity:	97.75% as reported in MRID 43914901 for this lot number.
CAS # of TGAI:	2122-70-5

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at dosing:	Young adults; Males: 2.100-2.775 g, females: 2.475-2.750 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:	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 14, 1994; End: July 28, 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-naphthaleneacetic acid, ethyl ester 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 necropsies were performed at the end of the study.

TABLE 1. Doses, mortality/animals			
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: Decreased defecation, diarrhea, soft feces, and/or nasal discharge were noted from a few males and females starting on day 1 or later with recovery by day 12. Erythema was noted on 3/10, 5/10, and 3/10 animals on days 1, 3, and 7, respectively. Desquamation was noted on 2/10, 1/10, 5/10, and 1/10 animals on days 3, 7, 10, and 14, respectively.

C. BODY WEIGHT: All animals gained weight during the study.

D. NECROPSY: One female had the small intestine filled with a green liquid and one female had the large intestine filled with gas.

E. REVIEWER'S CONCLUSIONS: Technical 1-naphthaleneacetic acid, ethyl ester is in EPA **Dermal Toxicity Category III**.

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

I

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT
[OPPTS 870.2400 (§81-4) OECD 405]
MRID 43494104

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. 03-22

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: JUL 04 2003

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

Signature: H T Borges
Date: JUL 04 2003

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

Signature: Robert H. Ross
Date: JUL 04 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 04 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#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D210701
SUBMISSION NO.: S315600, S511187

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetic acid, ethyl ester (97.75%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetic acid, ethyl ester - Primary eye irritation study in rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Laboratory Study No. 1354-94, August 30, 1994. MRID 43494104. Unpublished. 17 pages.

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

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 43494104), 0.1 mL by volume of technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) 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 were not noted on any rabbit during the study. One male had positive conjunctival irritation (redness) one hour after test material instillation with resolution by 24 hours. The highest maximum mean total score was 6.0, recorded one hour after test material instillation.

In this study, Technical 1-naphthaleneacetic acid, ethyl ester was minimally irritating to the eye based on the highest maximum mean total score (6.0) recorded one hour after test material instillation. The test material is in EPA Toxicity Category IV.

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-naphthaleneacetic acid, ethyl ester

Description:	Clear, colorless to light brown liquid
Lot/Batch #:	AM 315002
Purity:	97.75% as reported in MRID 43914901 for this lot number..
CAS # of TGAI:	2122-70-5

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

3. **Test animals:**

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at dosing:	Young adults (3-6 months); 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:	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 11, 1994; End: July 14, 1994

2. **Animal assignment and treatment:** The undiluted test material (0.1 mL) 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 were not noted on any rabbit during the study. One male had positive redness (grade 2) one hour after test material instillation with resolution by 24 hours. The highest maximum mean total score was 6.0 recorded one hour after test material instillation.

This classifies the test material as minimally irritating. Technical 1-naphthaleneacetic acid, ethyl ester is in **TOXICITY CATEGORY IV.**

B. REVIEWER'S CONCLUSIONS: Technical 1-naphthaleneacetic acid, ethyl ester is classified in **TOXICITY CATEGORY IV** for eye irritation.

C. DEFICIENCIES: None.

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG
[OPPTS 870.2600 (§81-6) OECD 406]
MRID 43494105

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. 03-22

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: JUL 04 2003

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

Signature: H T Borges
Date: JUL 04 2003

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

Signature: Robert H. Ross
Date: JUL 04 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 04 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** 9/4/03

Template version 11/01

TXR#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D210701
SUBMISSION NO.: S315600,

TEST MATERIAL (PURITY): Technical 1-Naphthaleneacetic acid, ethyl ester (97.75%)

SYNONYMS: Not reported

CITATION: Kuhn, J. (1994) Technical 1-Naphthaleneacetic acid, ethyl ester - Guinea pig maximization test for topically applied test material. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Laboratory Study No. 1355-94, November 9, 1994. MRID 43494105. Unpublished. 19 pages.

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

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 43494105) with 5% v/v technical 1-naphthaleneacetic acid, ethyl ester (Lot No. AM 315002; 97.75% a.i. as reported in MRID 43914901 for this lot) 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 after challenge.

In this study, Technical 1-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester

Description:	Clear, colorless to light brown liquid
Lot/Batch #:	AM 315002
Purity:	97.75% as reported in MRID 43914901 for this lot number
CAS # of TGAI:	2122-70-5

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

3. **Test animals:**

Species:	Guinea pigs	
Strain:	Hartley	
Age/weight at start:	Age not reported; males: 393-461 g; females: 360-423 g	
Source:	SASCO Inc., Madison, WI	
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: August 2, 1994; End: August 25, 1994

2. **Animal assignment and treatment:** The animals were induced and challenged according to the Magnusson-Kligman Maximization Test. The upper back and 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, the undiluted test material 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 undiluted test material 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 (20/20) at the sites injected with solution containing Freund's complete adjuvant, but not on the sites with test materials in vehicle control. Nine of ten control animals had erythema with or without edema 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-naphthaleneacetic acid, ethyl ester was not a sensitizer.

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

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-naphthaleneacetic acid, ethyl ester was not a sensitizer in this test.

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

K

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER (1-NAAEt)

Study Type: 82-1a; A 90-Day Oral (Feeding) Toxicity Study of 1-Naphthaleneacetic Acid, Ethyl Ester (1-NAAEt) in the Rat

Work Assignment No. 1-34A (MRID 43896002)

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
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Joan Harlin, M.S.

Signature: Joan Harlin
Date: 6/18/96

Secondary Reviewer
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 6/18/96

Project Manager
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 6/19/96

Quality Assurance
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 6/18/96

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

TXR # 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D223222 **SUBMISSION CODE**: S5500657

P.C. CODE: 056008 **TOX. CHEM. NO.**: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester (100% a.i.)

SYNONYMS: 1-NAAET

CITATION: Trimmer, G.W. (1995) 90-Day subchronic oral toxicity study in the rat with 1-Naphthaleneacetic Acid, Ethyl Ester (1-NAAET) (MRD-94-835). Exxon Biomedical Sciences, Inc., Toxicology Laboratory, Mettlers Road, CN 2350, East Millstone, NJ 08875-2350. Laboratory Project ID 183570. 295 pages. November 15, 1995. MRID 43896002. Unpublished.

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

EXECUTIVE SUMMARY:

In a subchronic oral toxicity study (MRID 43896002), 1-naphthaleneacetic acid, ethyl ester (Lot # AM 315002; 100% ai) was administered, in the diet, to CRL:CD BR rats (10/sex/dose) at dose levels of 400, 2000 or 8000 ppm for 13 weeks. The actual average doses at the end of the study were 19-25 mg/kg/day for the 400 ppm group, 92-123 mg/kg/day for the 2000 ppm group, and 388 - 519 mg/kg/day for the 8000 ppm group, for males and females, respectively.

Lower body weight, body weight gain, food consumption and food efficiency were observed for the 8000 ppm males and females compared to the controls. Body weights for the males were 7-13% lower and for the females were 9-21% lower than the corresponding controls throughout the study. Body weight gains were significantly reduced for both sexes at various weekly intervals throughout the study, and by the end of the study, were 18 and 38% lower for males and females, respectively, than the control gains. Mean food consumption by the males and females was 5-11 and 15-22 lower, respectively, than the control values for most weekly intervals; decreased food efficiency for both sexes was observed at most weekly intervals.

Increased relative and/or absolute liver and kidney weights were observed for the 2000 and 8000 ppm treatment groups. Relative liver weights were 21% higher for the 2000 ppm

females and 20 and 58% higher for the 8000 ppm males and females, respectively, compared to the control weights. Absolute liver weights were 13 and 24% higher for the 2000 and 8000 ppm females, respectively, compared to the controls. Relative kidney weights were higher for both sexes from the 2000 ppm (11% higher) and 8000 ppm (17-23% higher) treatment groups. Absolute kidney weight for the 2000 ppm males was 16% higher than the controls but was not increased for the 8000 ppm males. No associated macroscopic or microscopic changes were observed in the livers and kidneys of rats from any treatment group. Decreased red blood cell counts, hemoglobin, and hematocrits for both sexes from the 2000 and 8000 ppm groups were not considered clinically significant, but appeared to be treatment-related since they were dose-dependent. The 8000 ppm males and females also exhibited increased total bilirubin (19-21% higher) in conjunction with reduced red blood cell counts, hemoglobin, and hematocrits.

No other treatment-related effects were observed during the study. No rats died during the study. No differences in clinical signs, ophthalmology, macroscopic or microscopic pathology were observed between any of the treatment and control groups. Decreased urine protein in the 2000 and 8000 ppm males was not observed in the corresponding females.

The **LOAEL** for this study is 8000 ppm (594 mg/kg/day) for male and female rats, based on lower body weight, suppressed body weight gain, and reduced food consumption as compared to the controls. Absolute and/or relative liver and kidney weights for both sexes were seen at this dose but were not accompanied by any macroscopic or microscopic changes, however, males and females at this dose also exhibited increased total bilirubin (19-21% higher) in conjunction with reduced red blood cell counts, hemoglobin, and hematocrits. The **NOAEL** is 2000 ppm (144 mg/kg/day) for both sexes.

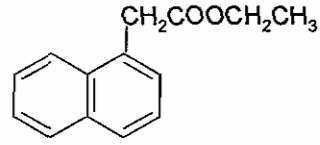
This 90-day oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodent species.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetic Acid, Ethyl Ester (1-NAAEt)
   Description: Brown Liquid
   Lot/Batch #: AM 315002
   Purity: 100% a.i.
   Stability of compound: Not provided
   CAS #: 2122-70-5
   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 - 158.5-193.5 g for males; 141.9-178.9 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° F
      Humidity: 40-70%
      Air changes: Not reported
      Photoperiod: 12 Hour light/12 hour dark cycle
   Acclimation period: 12 Days

B. STUDY DESIGN:

1. **In life dates** - Start: 8/23/94 End: 11/23/94

2. **Animal assignment**

   Animals in the original stock that were in poor health or had abnormalities were excluded from the selection process. Eighty rats (40/sex) were assigned to the test groups in Table 1 using a computer-generated randomization procedure which ensured homogeneity of group means and variances for body weight.

Table 1. Study design

Test Group	Conc. in Diet (ppm)	Nominal Dose to Animals (mg/kg/day)	Animals Assigned	
			Male	Female
1. Control	0	0	10	10
2. Low	400	40	10	10
3. Mid	2,000	200	10	10
4. High	8,000	800	10	10

3. Dose selection rationale

Dose selection was based on the results of a 14-day palatability study, which was not provided for review nor referenced. Based on the results of the palatability study, the dose levels selected for this subchronic oral toxicity study were 8000 ppm to establish the maximum tolerated dose, 2000 ppm as the intermediate dose, and 400 ppm to establish a no observed adverse effect level (NOAEL) for toxicity.

4. Diet preparation and analysis

The treated diet was prepared weekly by thoroughly mixing appropriate amounts of the test substance into the feed to ensure homogeneity. The mixtures were stored at room temperature. To determine homogeneity, samples from the 400 and 8000 ppm treatment feed were collected from the top, middle, and bottom of the bottles and analyzed on 8/23/94. To determine concentration, samples from the 400, 2000 and 8000 ppm treatment feed were collected and analyzed on Weeks 1, 5, 9, and 13. To determine stability, portions of the original day 0 samples from the 400 and 8000 ppm treatment feed were stored at ambient temperature or frozen and analyzed on Days 4, 8, 14, and 16 (400 ppm only).

Results

Homogeneity Analysis: (raw data provided in Table 2, page 293 of the study report):
400 ppm, 93.2-97.5% nominal
8000 ppm, 96.8-98.5% nominal

Stability Analysis (raw data provided in Table 4, page 295 of the study report):
Room temperature:
400 mg/kg/day: 92.2-109.0 % of nominal up to 16 days
8,000 mg/kg/day: 91.8-98.0 % of nominal up to 14 days
Frozen:
400 mg/kg/day: 88.5-96.0% of nominal up to 14 days
8,000 mg/kg/day: 93.0-98.0% of nominal up to 14 days

Concentration analysis: (raw data provided in Table 3, page 294 of the study report): (1, 5, 9, and 13 weeks):
400 mg/kg/day: 95.8-100.8% of nominal
2,000 mg/kg/day: 98.0-99.5% of nominal

8,000 mg/kg/day: 98.0-100.1% 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 - All animals were examined daily for signs of toxicity, including the nature, onset, severity, and duration of these effects.

c. Neurological Evaluations - See clinical observations above.

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 body weight/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 was calculated as mg food/kg body weight/day.

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
	Osmolality		

7. Sacrifice and Pathology

All test animals were sacrificed via exsanguination 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*
X	Trachea*	XX	Ovaries*+	X	parathyroids*
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 differences in clinical signs were observed between the treatment and control groups.

3. Neurological Evaluations - See clinical signs above.

B. BODY WEIGHT AND WEIGHT GAIN

Body weights for the 8000 ppm males and females were significantly ($p < 0.05$ or < 0.01) lower compared to the controls throughout the study, and were 7-13% and 9-21% lower for the males and females, respectively, compared to the control weights. In the 8000 ppm treatment groups, body weight gains were significantly ($p < 0.05$ or < 0.01) reduced for males during Weeks 1-3, 6, and 11, and for females during Weeks 1, 2, 4, and 5. By the end of the study, body weight gains for the 8000 ppm males and females were approximately 18 and 380 lower than the respective control weight gains (Table 3). Body weights and body weight gains for the 400 and 2000 ppm males were not significantly different from the controls throughout the study. The reduced body weight gain for the 400 ppm females during Weeks 2 and 5 ($p < 0.05$ or < 0.01) was not observed at other weekly intervals, and therefore was not considered to be treatment-related. Final body weight gains for the 400 and 2000 ppm females were 12 and 9% lower than the control gain (Table 3). These lower body weight gains compared to the control gain were most likely a reflection of the high final body weight gain for the controls, rather than a suppression of body weight gain due to treatment. It was noted that several control females exhibited higher body weight gains compared to the other control females throughout the study, which increased the average body weight gain for the group.

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)			Body Weight Gain (% of Control)[c]
	0 Days	90 Days[b]	0-28 Days	0-49 Days	0-90 Days[b]	
Males						
0	172.5±9.2	622.2±47.0	131.6	341.5	449.7	---
400	172.9±9.4	628.8±57.7	240.2	346.2	455.9	+1.4
2,000	173.4±8.4	649.1±59.4	243.1	351.3	475.7	+5.8
8,000	172.5±6.8	539.5±50.5**	192.0	281.3	367.0	-18.4
Females						
0	156.5±8.7	327.9±24.7	97.5	137.0	171.4	----
400	158.5±10.1	308.9±28.4	89.8	124.0	150.4	-12.2
2,000	157.3±7.4	313.9±27.3	89.9	127.1	156.6	-8.6
8,000	155.5±8.3	261.7±21.0**	55.8	84.3	106.2	-38.0

[a] Data obtained from Tables 2, pages 50-51, in the study report.
[b] Final body weights for female rats were recorded on Day 91 of the study.
[c] Calculated by the reviewer.
* Significantly different ($p < 0.01$) from the controls.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - Weekly mean food consumption by the 8000 ppm males was 8.40 lower than the controls during Week 1 and 5-110 lower during Weeks 5-13; the difference was significant ($p < 0.05$ or < 0.01) during Weeks 1 and 1113. Weekly mean food consumption by the 8000 ppm females was 15-22% lower than the controls at all weekly study intervals ($p < 0.05$ or < 0.01). Weekly mean food consumption by the 400 and 2000 ppm males and females was comparable to the corresponding controls throughout the study (Table 4).

TABLE 4. WEEKLY MEAN (G/ANIMAL/DAY) DURING 13-WEEK STUDY.[a]

Test Group (ppm)	Weekly Mean Food Consumption (g)	
	Males	Females
0	172.3-223.6	137.0-147.6
400	178.2-234.6	128.5-140.4
2000	178.2-232.8	133.6-147.2
8000	156.1-206.8	111.0-119.2

a Data obtained from Table 4 pages 56-57, in the study report.

2. Compound consumption - Mean measured doses decreased for all groups over time. (Table 5).

TABLE 5. ACTUAL CONSUMPTION OF 1-NAPHTHALENEACETIC ACID, ETHYL ESTER DURING 13 WEEKS OF FEEDING.[a]

Test Group (ppm)	Nominal Dose to animal (mg/kg/day)	Actual Average Consumption of Test Substance (mg/kg/day)	
		Males (Mean)	Females (Mean)
400	40	53-19 (29)	45-25 (31)
2000	200	257-92 (144)	231-123 (156)
8000	800	948-388 (594)	780-519 (594)

[a] Data obtained from page 28 of the study report,

3. Food efficiency - Males and females in the 8000 ppm groups had lower food efficiency during the entire study; the differences were significant ($p < 0.01$) for males during Weeks 1-3 and 6 and for females during Weeks 1 and 2. Food efficiency in the 400 and 2000 ppm treatment groups was comparable to the controls throughout the study.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ophthalmoscopic abnormalities were noted. At study termination, focal retinopathy was observed in one control female, one female from each treatment group, one 2000 ppm male, and two 8000 ppm males. These lesions were reported to be "typical of rats of this age and strain and were not considered treatment-related" [page 28].

E. BLOOD ANALYSIS

1. Hematology - The 2000 and 8000 ppm treatment groups exhibited decreases in red blood cell counts, hemoglobin, and hematocrit values. These decreases were significant ($p < 0.05$ or < 0.01) for red blood cell counts in females (5% lower), hemoglobin in males (3-4% lower) and females (6-9% lower), and hematocrits for males (4-5% lower) and females (7-8% lower) compared to the controls (Table 6). The study author stated that since these differences were <9% of the control values, they were not considered to be clinically significant [page 291. However, although these differences may not be clinically significant, they appeared to be treatment-related since the decreases were dose-related, but are considered not adverse because these decreased are less than 9% of the control vlaues. The 8000 ppm females had significantly ($p < 0.05$) decreased mean corpuscular hemoglobin (MCH; 17.9 pg vs. 18.7 pg for controls) that was not observed in other treatment groups, and decreased numbers of eosinophils; no other white blood cell counts were significantly different from the controls. The study author stated that the individual white blood cell counts and red blood cell morphology for the treatment groups "appeared unremarkable" [page 29]. No treatment-related differences in hematological parameters were observed between the 400 ppm treatment groups and the control groups.

TABLE 6. RED BLOOD CELL, HEMOGLOBIN, AND HEMATOCRIT LEVELS IN MALE AND FEMALE RATS.[a]

Conc. inDiet (ppm)	RBC (10^6/mm^3)	Hemoglobin (g/dL)	Hematocrit (%)
Male			
0	8.40 ± 0.40	14.9 ± 0.4	40.9 ± 0.8
400	8.16 ± 0.35	14.6 ± 0.6	39.8 ± 1.5
2,000	8.14 ± 0.29	14.4* ± 0.4	39.3* ± 1.1
8,000	8.13 ± 0.24	14.3* ± 0.6	39.0** ± 1.2
Female			
0	7.91 ± 0.35	14.8 ± 0.4	40.5 ± 0.9
400	7.71 ± 0.36	14.5 ± 0.6	39.3 ± 1.7
2,000	7.52* ± 0.19	13.9** ± 0.5	37.7** ± 1.2
8,000	7.55* ± 0.34	13.5** ± 0.5	37.3** ± 1.5

a Data obtained from Table 8, page 66, in the study report.
* Significantly different ($p < 0.05$) from the controls.
** Significantly different ($p < 0.01$) from the controls.

2. Clinical Chemistry - In the 8000 ppm treatment groups, total bilirubin was 18.9% higher in males and 20.7% higher in females compared to the respective controls (Table 7). The increased bilirubin levels in both sexes may be related to dose-related decreases in red blood cell count, hemoglobin, and hematocrit values. The 8000 ppm males had 4.8% lower total protein ($p < 0.01$) compared to the controls; decreased total protein was not observed in the 8000 ppm females. Decreased glucose levels in the males (18.2% lower; $p < 0.01$) and females (12.6% lower; not statistically significant) were likely due to significantly decreased food consumption. In the 8000 ppm females only, significantly increased triglyceride (39% higher than controls; $p < 0.05$) and decreased chloride (3.2% lower than control; $p < 0.01$) levels were observed. No treatment-related differences in clinical chemistry parameters were observed between the 400 and 2000 ppm treatment groups and the controls. Decreased creatine (0.4 mg/dL vs. 0.5 mg/dL for controls; $p < 0.05$) observed in the 2000 ppm males was not observed in the 8000 ppm males.

TABLE 7. SELECTED MEAN CLINICAL CHEMISTRY PARAMETERS FOR CONTROL AND TREATED MALE AND FEMALE RATS.[a]

(ppm)	Glucose (mg/DL)	Total Bilirubin (mg/dL)	Triglycerides (mg/dL)	Total Protein g/dL	Chloride (mmole/L)
Male					
0	158.1 ± 27.0	0.53 ± 0.08	57 ± 27	6.2 ± 0.1	108.3 ± 2.0
400	143.8 ± 13.9	0.54 ± 0.07	47 ± 22	6.0 ± 0.1	108.9 ± 2.0
2,000	137.6 ± 8.6	0.52 ± 0.13	57 ± 20	6.0 ± 0.2	107.3 ± 1.9
8,000	129.3** ± 11.9	0.63 ± 0.08	52 ± 18	5.9** ± 0.2	106.6 ± 1.8
Female					
0	141.7 ± 16.4	0.58 ± 0.10	23 ± 9	6.1 ± 0.3	110.8 ± 2.0
400	134.4 ± 14.5	0.62 ± 0.06	21 ± 4	6.4 ± 0.4	109.8 ± 1.6
2,000	127.1 ± 16.4	0.61 ± 0.07	22 ± 4	6.2 ± 0.4	108.6 ± 1.6
8,000	123.8 ± 11.9	0.70* ± 0.12	32* ± 10	6.1 ± 0.3	107.3** ± 2.5

a Data obtained from Table 8, page 69-70, in the study report.
* Significantly different ($p < 0.05$) from the controls.
** Significantly different ($p < 0.01$) from the controls.

F. URINALYSIS - Urine protein was decreased in the 2000 and 8000 ppm males, reaching statistical significance in the high-dose males only ($p < 0.05$). No other differences in urinalysis parameters were observed between the treatment and control groups.

TABLE 8. MEAN URINE PROTEIN FOR CONTROL AND TREATED MALE AND FEMALE RATS.[a]

Test Group (ppm)	Mean Urine Protein (mg/16 hrs)	
	Males	Females
0	24.3	3.7
400	23.9	3.3
2000	14.9	3.3
8000	10.3*	2.8

a Data obtained from Table 11, page 72, in the study report.
* Significantly different ($p < 0.05$) from the controls.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight - Increased absolute and/or relative liver and kidney weights were observed for the 2000 and 8000 ppm treatment groups; no associated macroscopic or microscopic changes were noted. Relative liver weights were increased by 21% for the 2000 ppm females, and by 20% and 58% for the 8000 ppm males and females, respectively, compared to the corresponding control weights ($p < 0.01$; Table 9). Absolute liver weights were significantly

($p < 0.05$ or < 0.01) increased by 13 and 24% for the 2000 and 8000 ppm females, respectively. Relative kidney weight was significantly ($p < 0.05$ or < 0.01) increased by 11% for the 2000 ppm males and females, and by 23 and 17% for the 8000 ppm males and females, respectively. Absolute kidney weight was significantly ($p < 0.05$) increased for the 2000 ppm males only, and was 16% higher than the control weight. The increased relative brain weight noted for the 8000 ppm females (22% higher than controls; $p < 0.01$) was likely due to the depressed body weights since the absolute organ weight did not differ from the control weight, and there were no associated macroscopic or microscopic changes. No other significant differences were observed between organ weights of the treated and control females.

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

Conc. in Diet (ppm)	Terminal Body Weight[b] (g)	Liver Weight		Kidney Weight	
		Absolute (g)	Relative	Absolute (g)	Relative
Male					
0	589.9	15.11	0.025	3.86	0.0066
400	593.5	15.90	0.027	4.26	0.0072
2,000	614.0	16.58	0.027	4.49*	0.0073*
8,000	509.9**	15.22	0.030**	4.15	0.0081**
Female					
0	307.3	7.40	0.024	2.20	0.0072
400	288.4	7.36	0.026	2.16	0.0075
2,000	294.8	8.39*	0.029**	2.36	0.0080**
8,000	242.8**	9.18**	0.038**	2.04	0.0084**

[a] Data were obtained from Table 12 and 13, pages 74-75 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 differences in gross pathology were observed between the treatment and control groups. Isolated instances of small adrenal glands, parasite scabs, dilated pelvis, discolored epididymis, and maloccluded/broken incisors were observed in the control and/or treatment groups.

3. Microscopic pathology

a. Non-neoplastic -No microscopic non-neoplastic pathological differences were observed between the treatment and control groups. Observed microscopic alterations were reported to be "common spontaneously occurring changes in young laboratory rats and the incidence or intensity of these changes was not influenced by administration of the test material" [page 32 of the study report]. These changes included foci of mononuclear inflammatory cells in all test groups, periportal

hepatocellular vacuolation in females from all test groups, and lymphoplasmacytic hyperplasia in the mesenteric and/or mandibular lymph nodes in most rats from the control and 8000 ppm groups. The author stated that lymphoplasmacytic hyperplasia commonly occurs spontaneously in young laboratory rats of this age and strain [page 32].

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 the **LOAEL** for both sexes was 8000 ppm, based on lower body weight, suppressed body weight gain, and reduced food consumption as compared to the controls. The **NOAEL** was 2000 ppm.

C. Reviewer's Discussion

Lower body weight, body weight gain, food consumption and food efficiency were observed for the 8000 ppm males and females compared to the controls. Body weights for the males were 7-13% lower and for the females were 9-21% lower than the corresponding controls throughout the study. Body weight gains were significantly reduced for both sexes at various weekly intervals throughout the study, and by the end of the study, were 18 and 38% lower for males and females, respectively, than the control gains. Mean food consumption by the males and females was 5-11 and 15-22 lower, respectively, than the control values for most weekly intervals; decreased food efficiency for both sexes was observed at most weekly intervals.

Increased relative and/or absolute liver and kidney weights were observed for the 2000 and 8000 ppm treatment groups. Relative liver weights were 21% higher for the 2000 ppm females and 20 and 58% higher for the 8000 ppm males and females, respectively, compared to the control weights. Absolute liver weights were 13 and 24% higher for the 2000 and 8000 ppm females, respectively, compared to the controls. Relative kidney weights were higher for both sexes from the 2000 ppm (11% higher) and 8000 ppm (17-23% higher) treatment groups. Absolute kidney weight for the 2000 ppm males was 16% higher than the controls but was not increased for the 8000 ppm males. No associated macroscopic or microscopic changes were observed in the livers and kidneys of rats from any treatment group. Decreased red blood cell counts, hemoglobin, and hematocrits for both sexes from the 2000 and 8000 ppm groups were not considered clinically significant, but appeared to be treatment-related since they were dose-dependent. The 8000 ppm males and females also exhibited increased total bilirubin (19-21% higher) in conjunction with reduced red blood cell counts, hemoglobin, and hematocrits.

No other treatment-related effects were observed during the study. No rats died during the study. No differences in clinical signs, ophthalmology, macroscopic or microscopic pathology were observed between any of the treatment and control groups. Decreased urine protein in the 2000 and 8000 ppm males was not observed in the corresponding females.

The **LOAEL** for this study is 8000 ppm (594 mg/kg/day) for male and female rats, based on lower body weight, suppressed body weight gain, and reduced food consumption as compared to the controls. Absolute and/or relative liver and kidney weights for both sexes were seen at

this dose but were not accompanied by any macroscopic or microscopic changes, however, males and females at this dose also exhibited increased total bilirubin (19-21% higher) in conjunction with reduced red blood cell counts, hemoglobin, and hematocrits. The **NOAEL** is 2000 ppm (144 mg/kg/day) for both sexes.

B. STUDY DEFICIENCIES

No deficiencies were noted in this study.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - non-rodents (870.3150)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range ppm : diet	Doses ppm: diet	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
056008	43896002	subchronic	rat	90	oral	diet	400-8000	0, 400, 2000, 8000	144	594	Body weight, liver and kidney increased weight and clinical chemistry	Toxicity

L

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

Study Type: 82-1b; A 13-Week Oral (Capsule) Toxicity Study of 1-Naphthaleneacetic Acid, Ethyl Ester (1-NAAEt) in the Beagle Dog

Dynamac Study No. 1-34B (MRID 43914901)

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
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Kathleen Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 5/20/96

Secondary Reviewer
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 5/20/96

Project Manager
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 6/6/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 6/6/96

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 # 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D223222 **SUBMISSION CODE**: S5500657

P.C. CODE: 056008 **TOX. CHEM. NO.**: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester Technical (97.75%)

SYNONYMS: 1- NAAEt

CITATION: Farrell, D. (1995) A 13-week subchronic oral (capsule) toxicity study of 1-Naphthaleneacetic Acid, Ethyl Ester Technical in the beagle dog. Bio-Research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, Canada, H9X 3R3. Laboratory Project ID 86452. 334 pages. October 4, 1995. MRID 43914901. Unpublished.

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

EXECUTIVE SUMMARY:

In a subchronic oral toxicity study (MRID 43914901), 1-naphthaleneacetic acid, ethyl ester (Lot/Batch # AM 315002; 97.75% ai) was fed (gelatin capsules) to beagle dogs (4/sex/dose) at dose levels of 0, 40, 125, or 400 mg/kg/day for 13 weeks.

All dogs survived the treatment. Treatment-related clinical signs were limited to soft or liquid feces particularly in males and to a lesser extent in females at the high dose of 400 mg/kg/day. Male dogs in the 40, 125, or 400 mg/kg/day treatment groups had a 4- to 5.5-fold increase in soft/liquid feces (maximum 65 instances during 13 weeks in the 400 mg/kg/day group) compared to the control group. For females, there was one incident of soft/liquid feces in the control group, five in the 40 mg/kg/day group, eight in the 125 mg/kg/day group, and 19 in the 400 mg/kg/day group during the 13-week study.

A treatment-related lower body weight gain (20% less than the control) was seen in the males and females of the high dose group. Males in the 40 or 125 mg/kg/day treatment group were 25-29% heavier, and males in the 400 mg/kg/day treatment group were 20% lighter than males in the control group; all three groups consumed 19% more food than the control during the study. Female dogs in the 400 mg/kg/day treatment groups consumed 11% less food over the course of

the study.

Male dogs in the 40, 125, or 400 mg/kg/day treatment groups had significantly ($p < 0.05$ or 0.01) lower red blood cell, hemoglobin, and hematocrit levels, and lower mean platelet volumes (MPV) than the controls throughout the study (Table 4). In the high dose males at 12 weeks, these were 18%, 17%, 17% and 22% lower than the controls for RBC, hemoglobin, hematocrit and MPV levels, respectively. Although , these parameters were within the historical range values for this dog type, they were at the lower end of the range and may suggest anemic affects caused by the administration of this material at this dose. Low white blood cell counts in female dogs in the 125 or 400 mg/kg/day treatment groups after 12 weeks of treatment were also within expected biological ranges.

No other treatment-related responses were observed during the study. No differences were observed in clinical blood chemistry, ophthalmology, urine volume or chemistry, organ weights, or macroscopic or microscopic organ morphology between dogs in the treated and the control groups. No neoplastic tissue was observed. The **LOAEL** for this study is 400 mg/kg/day, based on soft/liquid feces and the depressed body weight gains of male and female dogs at this treatment level. Additionally some blood parameters (RBC, hemoglobin, hematocrit and mean platelet volume) were all depressed in the male dogs at this level. The **NOAEL** was 125 mg/kg/day.

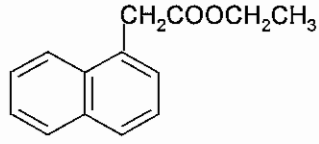
This 90-day oral toxicity study in the dog is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in non-rodent species.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 1-Naphthaleneacetic Acid, Ethyl Ester Technical (1-NAAEt)
   Description: Liquid
   Lot/Batch #: AM 315002
   Purity: 97.75% a.i.
   Stability of compound: The expiration date of the test material was reported to be May 15, 1997.
   CAS #: 2122-70-5
   Structure:



2. **Vehicle and/or positive control**: Gelatin capsules

3. **Test animals**:
   Species: Dog
   Strain: Beagle
   Age and weight at study initiation: Approximately 4-6 months of age; body weight range - 6.1 - 7.8 kg for males; 5.3 - 6.8 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: 28 Days

B. STUDY DESIGN:

1. **In life dates** - Start: 1/26/95 End: 4/28/95

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

Table 1. Study design.

Test Group	Dose to Animals (mg/kg/day	Animals Assigned	
		Male	Female
1 Control	0	4	4
2 Low	40	4	4
3 Mid	125	4	4
4 High	400	4	4

3. Dose selection rationale

Dose selection was based on the results of two studies using 1-naphthaleneacetic acid, ethyl ester: an acute oral toxicology study in which the test substance was administered once at 100, 200, or 400 mg/kg/day; and a short-term study in which it was administered daily for 14 days at 150 or 350 mg/kg/day. The study author reported that "red material" was present in the stools of the males treated once at 200 and 400 mg/kg/day, and that "black stools" were noted in females treated for 14 days at 350 mg/kg/day. No gross pathological abnormalities were found in animals treated for 14 days at 150 or 350 mg/kg/day. No additional information about these studies was provided.

4. Diet Preparation and Analysis

Doses were prepared daily, and were based on the most recently recorded body weights (dogs were weighed weekly). Liquid 1-naphthaleneacetic acid, ethyl ester, was dispensed into a size 12 gelatin capsule, which was then placed inside a size 11 capsule. Filled capsules were stored at room temperature in the dark until use. Animals were dosed at the same time each day, 7 days each week, for a minimum of 13 weeks. Control animals were given the same number of capsules as the high dose animals.

4. 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.

C. METHODS:

1. Observations:

All animals were observed twice daily for mortality and abnormal clinical signs. Each animal was given a physical examination at least once each week.

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 for each animal was determined weekly, beginning 2 weeks prior to the initial dosing and continuing throughout the treatment period. Food consumption was reported as g food/animal/day.

4. Ophthalmoscopic examination:

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) exams were conducted on all animals prior to the initial dosing and during week 12 of dosing.

5. Hematology & Clinical Chemistry:

Blood was collected from all animals prior to the initial dosing and during weeks 4, 8, and 12 of dosing. Blood was collected from the "appropriate" vein in the morning following overnight fasting and, if applicable, prior to dosing. The CHECKED (X) parameters were examined in all samples analyzed.

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*
	ENZYMES	X	Total bilirubin
		X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	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 animals that died and those sacrificed on schedule were subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination and 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)*T
X	Stomach*				
X	Duodenum*	X	Lymph nodes*	XX	Pituitary*
X	Jejunum*	XX	Spleen*	X	Eyes (optic n.)*T
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*++
		XX	Ovaries*+	XX	Parathyroids*++
X	Trachea*		Oviducts		
X	Lung*	X	Uterus*		OTHER
	Nose		Vagina		
	Pharynx			X	Bone*
	Larynx				Skeletal muscle*
	Tonsils			X	Skin*
				X	All gross lesions and masses*

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

II. RESULTS

A. Observations:

1. Mortality - No dogs died during the study.

2. Clinical Signs -

Soft/liquid feces were observed in males in the control and all treatment groups throughout the 13-week study (Appendix 1 of this report). This symptom was much more common in the treated males than the controls, but it was inconclusive whether the incidence of soft/liquid feces was related to concentration. During the entire 13-week period, there were 12 instances of soft/liquid feces in the control group, 48 in the 40 mg/kg/day group, 49 in the 125 mg/kg/day group, and 65 in the 400 mg/kg/day group. However, when considered on a weekly basis, during at least 10 weeks of the study the frequency of soft/liquid feces in the 40 or 125 mg/kg/day groups was equal to or higher than the frequencies in the 400 mg/kg/day group. For all male groups, the incidence of soft/liquid feces began to subside about week 8. Soft/liquid feces were observed in females in the 40, 125, and 400 mg/kg/day sporadically during weeks 1 through 9; the incidence of soft/liquid feces increased with increasing dose rate. During the 13-week study, there was 1 event in the female control group, 5 in the 40 mg/kg/day group, 8 in the 125 mg/kg/day group, and 20 in the 400 mg/kg/day group. Vomiting and other clinical signs occurred randomly and sporadically in all treatment groups.

B. Body weight and weight gain

Male and female dogs in the 400 mg/kg/day treatment groups had lower body weights and body weight gains than the controls and other treatment groups (Table 2). These mean differences were not statistically significant, probably because the within-group variability in body weights was high for the small sampling size.

TABLE 2. BODY WEIGHTS AND BODY WEIGHT GAINS (KG) AT 13 WEEKS [a]

Dose level mg/kg/day	Initial body weight, kg	13-week body weight, kg	Body weight gain, % of control
Males			
0	6.98 ± 0.54	9.35 ±0.71	--
40	7.30 ± 0.39	10.48 ± 0.96	128.7
125	6.58 ± 0.32	9.65 ± 0.21	124.3
400	6.72 ± 0.53	8.80 ± 1.15	84.2
Females			
0	6.05 ± 0.58	8.08 ± 0.93	--
40	6.43 ± 0.25	8.45 ± 0.60	99.5
125	6.25 ± 0.31	8.27 ± 1.03	99.5
400	6.13 ± 0.33	7.75 ± 0.72	79.8

a Data obtained from Tables 2 and 3, pages 33 and 34, and Appendix 2, pages 132-140, in the study report.

C. Food consumption

Weekly mean food consumption by male dogs in the 40, 125, and 400 mg/kg/day treatment groups was approximately 19% greater than the male controls (Table 3). The weekly food consumption difference for males was significant ($p < 0.05$ or 0.01) during 4-5 weeks of the 13-week study. Weekly mean food consumption by female dogs in the 40 and 400 mg/kg/day treatment groups was 10% greater and 11% lower, respectively, than the female controls.

TABLE 3. WEEKLY MEAN FOOD CONSUMPTION (G/ANIMAL/DAY).[a]

Sex	Dose level mg/kg/day			
	0	40	125	400
Male	306	362	363	365
Female	315	346	305	280

a Data calculated from Table 4, page 35, in the study

D. Ophthalmoscopic examination

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

E. BLOOD ANALYSIS:

1. Hematology -

Male dogs in the 40, 125, or 400 mg/kg/day treatment groups had significantly ($p < 0.05$ or 0.01) lower red blood cell, hemoglobin, and hematocrit levels, and lower mean platelet volumes (MPV) than the controls throughout the study (Table 4). In the high dose males at 12 weeks, these were 18%, 17%, 17% and 22% lower than the controls for RBC, hemoglobin, hematocrit and MPV levels, respectively. Although , these parameters were within the historical range values for this dog type, they were at the lower end of the range and may suggest anemic affects caused by the administration of this material at this dose. Low white blood cell counts in female dogs in the 125 or 400 mg/kg/day treatment groups after 12 weeks of treatment were also within expected biological ranges.

M

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER

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

Dynamac Study No. 1-30A (MRID 43581002)

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
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Kathleen Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 4/15/96

Secondary Reviewer
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/15/96

Project Manager
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/15/96

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date: 4/15/96

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.
RAB3, Health Effects Division (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D.
RRB4, Health Effects Division (7509C)

Signature: A. Khasawinah
Date Aug. 6, 2003
Signature: William Dykstra
Date 8/6/03
Template version 11/01

TXR#: 0051960

DATA EVALUATION RECORD

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

DP BARCODE: D213981 **SUBMISSION NO.**: S484982

PC CODE: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester (97.75%)

SYNONYM(S): Rootone, 1-NAAEt; MRD-94-835

CITATION: Trimmer, G.W. (1995) 21-Day Repeated Dose Dermal Toxicity Study in the Rat with 1-Naphthaleneacetic Acid, Ethyl Ester (MRD-94-835). Exxon Biomedical Sciences, Inc., Toxicology Laboratory, Mettlers Road, East Millstone, NJ, 08875-2350. Laboratory Project ID 183510B. 145 pages. March 2, 1995. MRID 43581002. Unpublished.

SPONSOR: AMVAC Chemical Corporation, Los Angeles, CA.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 43581002), 1-naphthaleneacetic acid, ethyl ester (Lot # 315002; 97.75% 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.

For all treatment groups, there were no clinical signs of toxicity, and body weights, body weight gains, and food consumption were similar to the controls. There were no differences in hematology parameters, clinical blood chemistry, organ weights, or macroscopic or microscopic organ morphology between rats in the treated and the control groups. No neoplastic tissue was observed. Ophthalmoscopic examinations and urinalysis were not performed during the study.

Treatment-related dermal irritation was seen in the treated skin of all animals exposed to 1-Naphthaleneacetic acid ethyl ester. As the dose level was increased, the incidence and/or severity of epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia, and dermal inflammation in skin from the treated areas increased in the treated rats compared to the controls. In general, the severity of the reactions increased from minimal/slight to slight/moderate with increasing dose rate.

No systemic responses were observed. Therefore, the **LOAEL** for systemic toxicity is >1000 mg/kg/day and the **NOAEL** for systemic toxicity is 1000 mg/kg/day. The **LOAEL** for dermal irritation is 100 mg/kg, based on the presence of treatment-related dermal irritation in the treated skin of rats in the 100, 300, and 1000 mg/kg treatment groups. No **NOAEL** for dermal irritation was established.

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.

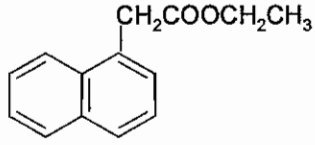
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 1-Naphthaleneacetic acid, ethyl ester (1NAAEt)

Description: Brown liquid
Lot/Batch #: Am 315002
Purity: 97.75% ai
Stability of compound: The test material was shown to be stable at room temperature for the duration of the study.

CAS #: 2122-70-5
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 267-297 g; Females - approximately 10 weeks, 231-261 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, *ad 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: 9/19/94 End: 10/10/94

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. The 1000 mg/kg is the limit dose for repeated dose dermal toxicity studies.

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. 1-Naphthaleneacetic acid, ethyl ester was applied "as received" to a 60 x 40 mm area on the clipped skin, then the treated area was covered with a moistened (reverse osmosis water) porous gauze dressing 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 -

4. Dermal Irritation - Erythema and edema were not observed in any animal.

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 386 - 396 g, and of female rats was 271-280 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. Although female rats in 100, 300, and 1000 mg/kg treatment groups had decreased mean serum alanine aminotransferase (ALT) activity compared to the control group, these decreased values were within expected ALT ranges, exhibited no treatment-related trend, and were statistically significant ($p < 0.05$) only for the 300 mg/kg treatment group. Decreases in the ALT activity are not considered toxicologically significant.

F. URINALYSIS:

Urine was not analyzed during the study.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight - No treatment-related differences in the relative or absolute 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 - Treatment-related epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia, and dermal inflammation were observed in skin from the treated areas (Table 2). In general, the severity of the reactions increased from minimal/slight to slight/moderate with increasing dose rate. The untreated skin of a few rats exhibited similar changes; these changes were not correlated to treatment rate, and were attributed to the procedures used to prepare the application sites and possible migration of the test substance during treatment to "untreated" areas. All other tissue abnormalities occurred randomly and sporadically in all study groups.

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

TABLE 2. HISTOMORPHOLOGICAL OBSERVATIONS IN THE TREATED AND UNTREATED SKIN OF RATS (total 5 rats/sex/dose).[a]

Observation/Severity	Males Dose (mg/kg/day)				Females Dose (mg/kg/day)			
	0	100	300	1000	0	100	300	1000
Treated Skin								
Epidermal hyperplasia/hyperkeratosis:								
Minimal	3	0	0	0	3	2	2	0
Slight	2	5	1	2	0	1	1	3
Moderate	0	0	4	3	0	1	2	2
Sebaceous glands hyperplasia:								
Minimal	1	1	0	2	1	2	3	0
Slight	1	3	3	2	0	1	2	5
Moderate	0	0	1	0	0	0	0	0
Dermal inflammation: Minimal	1	1	2	4	0	1	1	2
Untreated Skin								
Epidermal hyperplasia/hyperkeratosis:								
Minimal	1	2	3	0	0	0	0	1
Slight	0	1	1	0	0	0	0	0
Sebaceous glands hyperplasia:								
Minimal	0	0	1	0	0	0	0	0
Slight	0	1	1	0	0	0	0	0

[a] Data obtained from Table 1, Appendix K, page 112, in the study report.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that the **NOAEL** of 1-Naphthaleneacetic Acid, Ethyl Ester 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 conclusion that no systemic effects were observed in any treatment group. For all treatment groups, there were no clinical signs of toxicity. Body weights, body weight gains, and food consumption were similar to the controls. No differences were

observed in hematology parameters, clinical blood chemistry, organ weights, or macroscopic or microscopic organ morphology between rats in the treated and the control groups. No neoplastic tissue was observed. Ophthalmoscopic examinations and urinalysis were not performed during the study.

Treatment-related dermal irritation was seen in the treated skin of all animals exposed to 1-Naphthaleneacetic acid ethyl ester. Although epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia, and dermal inflammation were observed in the skin of rats from all treatment groups, the incidence and severity of these reactions increased as the dose level was increased from 0 to 100 to 300 to 1000 mg/kg.

Therefore, the **LOAEL** for systemic toxicity is >1000 mg/kg/day and the **NOAEL** for systemic toxicity is 1000 mg/kg/day. The **LOAEL** for dermal irritation is 100 mg/kg, based on the presence of treatment-related dermal irritation in the treated skin of rats in the 100, 300, and 1000 mg/kg treatment groups. No **NOAEL** for dermal irritation was established.

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
056008	43581002	subchronic	rat		dermal	dermal	0-1000	0, 100, 300, 1000	<100	100	Dermal irritation	No systemic toxicity

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 # 0051960

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: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester Technical (97.75%)

SYNONYM(S): None

CITATION: San, R.H.C. and M.L. Klug (1994) Salmonella Plate Incorporation Mutagenicity Assay (Ames Test) With a Confirmatory Assay; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU53.501001; 56 pages. Study Completion Date: December 2, 1994. MRID: 43581004. Unpublished

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In a microbial/mammalian microsome plate incorporation mutagenicity study (MRID 43581004), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to five doses of 1-naphthaleneacetic acid, ethyl ester technical (Lot # AM 315002; 97.75%) ranging from 33 to 5000 µg/plate with and without S9 activation. Based on analytical data, actual high doses used in the study were 4195 µg/plate (initial trial) and 3955 µg/plate (confirmatory trial). Two independently performed 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.

Cytotoxicity was observed for all strains at ≥3955 µg/plate -S9 and for the majority of strains at ≈1000 µg/plate -S9. In the presence of S9 activation, cytotoxicity was achieved at 4195 µg/plate. All strains responded in the expected manner to the appropriate positive control. **There was, however, no indication that 1-naphthaleneacetic acid, ethyl ester 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-Naphthaleneacetic acid, ethyl ester technical

Description: Amber liquid
Lot/batch number: AM 315002
Purity: 97.75%
Receipt date: June 10, 1994
Stability: Not reported
CAS number: 2122-70-5
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 ranged from 83.8-97.9% of the intended concentrations for the initial trial and from 79.1-101% of the target levels for the confirmatory trial. Based on these data, actual high doses used in the study were 4190 µg/plate (initial trial) and 3955 µg/plate (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. The test material was soluble at all tested levels. Severe cytotoxicity, as indicated by a marked reduction in the background lawn of growth and histidine revertants (his^+), was observed at the two highest doses (3333 and 5000 µg/plate) with or without S9 activation. His^+ revertant colonies were also reduced (≈≥56% of control) at 667 and 1000 µg/plate -S9. The remaining concentrations with or without S9 activation were not cytotoxic. Based on these findings, the mutation assay was conducted with five doses ranging from 33 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 1 & 2). The results were as follows:

1. **Nonactivated conditions**: Data from the two independent mutation assays conducted under nonactivated conditions were in good agreement and indicated that the test material was cytotoxic for all strains at 5000 µg/plate and for the majority of strains at 1000 µg/plate. No appreciable increases in his^+ revertant colonies were, however, noted at any noncytotoxic level of the nonactivated test substance.

2. **S9-activated Conditions**: In the initial S9-activated trial, reductions in the background lawn of growth and/or decreased revertant colonies were seen at 5000 µg/plate. The response was not reproduced in the confirmatory phase of testing. Our reviewers believe that the lack of a reproducible cytotoxic response is probably related to the lower that intended concentration of 1-naphthaleneacetic acid, ethyl ester technical that was applied, particularly in the confirmatory (3950 µg/plate--actual vs. 5000 µg/plate--intended). There was, however, no indication of a mutagenic effect in either trial.

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-acetic acid, ethyl ester 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 clearly cytotoxic doses without S9 activation (≈≥1000 µg/plate) and levels either at or near the cytotoxic concentration in the presence of S9 activation (>3955 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-naphthaleneacetic acid, ethyl ester 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 solutions (both trials), indicated an ≈16-20% difference between actual and target concentrations, it is not likely that this deficiency altered the outcome of the study as cytotoxicity was demonstrated at the highest dose for the majority of *S. typhimurium* strains.

Attachment 1. Reproduced from study report MRID 43581004, page 37

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article ID :Technical 1-NAPHTHALENEACETIC ACID, ETHYL ESTER
Study Number :G94AU53.501001 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation					
Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
Liver Microsomes : None					
0.0	29 ± 2	135 ± 5	14 ± 3	7 ± 2	8 ± 2
100	30 ± 1	130 ± 9	12 ± 2	8 ± 1	8 ± 4
333	29 ± 1	116 ± 8	17 ± 5	6 ± 2	11 ± 3
1000	24 ± 4	114 ± 16	10 ± 4	5 ± 2	8 ± 4
3333	21 ± 3	90 ± 10	10 ± 2	3 ± 1	4 ± 2
5000	12 ± 4	55 ± 13	5 ± 2	3 ± 1	0 ± 0
Pos	264 ± 6	881 ± 17	675 ± 18	1290 ± 143	514 ± 41
Liver Microsomes: Rat Liver S9					
0.0	41 ± 6	148 ± 7	15 ± 4	11 ± 2	13 ± 12
100	42 ± 7	168 ± 3	12 ± 4	9 ± 3	14 ± 4
333	38 ± 2	142 ± 10	16 ± 1	8 ± 2	17 ± 3
1000	44 ± 6	135 ± 14	13 ± 4	8 ± 1	12 ± 5
3333	41 ± 6	157 ± 18	12 ± 3	6 ± 1	7 ± 1
5000	36 ± 1	132 ± 4	8 ± 2	4 ± 3	3 ± 3
Pos	946 ± 38	790 ± 49	102 ± 6	105 ± 42	1335 ± 128

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

Attachment 2. Reproduced from study report MRID 43581004, page 38

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article ID :Technical 1-NAPHTHALENEACETAMIDE
Study Number :G94AU53.501001 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation					
Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
Liver Microsomes : None					
0.0	25 ± 3	164 ± 18	16 ± 6	9 ± 4	8 ± 1
100	27 ± 1	168 ± 16	14 ± 1	6 ± 1	8 ± 3
333	28 ± 6	138 ± 16	17 ± 12	5 ± 3	7 ± 3
1000	30 ± 7	152 ± 14	12 ± 2	6 ± 1	10 ± 2
3333	21 ± 5	118 ± 14	13 ± 2	4 ± 2	5 ± 3
5000	16 ± 4	70 ± 9	8 ± 3	2 ± 1	3 ± 2
Pos	333 ± 6	699 ± 33	511 ± 18	1332 ± 366	520 ± 26
Liver Microsomes: Rat Liver S9					
0.0	37 ± 12	175 ± 4	9 ± 3	8 ± 2	18 ± 17
100	36 ± 5	147 ± 37	16 ± 2	5 ± 3	18 ± 8
333	43 ± 8	167 ± 6	14 ± 1	6 ± 2	15 ± 4
1000	34 ± 9	166 ± 25	15 ± 3	6 ± 3	14 ± 6
3333	31 ± 6	214 ± 25	10 ± 2	3 ± 0	11 ± 4
5000	20 ± 7	208 ± 14	13 ± 3	5 ± 4	5 ± 2
Pos	2231 ± 265	2108 ± 227	174 ± 20	253 ± 46	1845 ± 223

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

P

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 # 0051960

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: 056008 TOX. CHEM. NO.: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester Technical (97.75%)

SYNONYM(S): None

CITATION: San, R.H.C. and Clark, J.J. 1995. L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay, Technical 1-Naphthaleneacetic Acid, Ethyl Ester:; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU53.702001; 41 pages. Study Completion Date: January 16, 1995. MRID : 43580201. Unpublished.

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In an in vitro mammalian cell forward gene mutation study (MRID No: 43580201), cultured L5178Y mouse lymphoma cells were exposed to doses of 1-naphthaleneacetic acid, ethyl ester technical (Lot # AM 315002; 97.75%) ranging from 10-100 µg/mL -S9 or 50-500 µg/mL +S9 (initial trial) and nonactivated doses of 20-100 µg/mL or S9-activated levels of 60-500 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, ≤25% viability) was seen at 100 µg/mL -S9 and ≤13% total viability was noted at ≥300 µg/mL +S9. The positive controls induced the expected response in the target cells in both trials. There was no evidence that the test material induced a mutagenic effect in the absence of exogenous metabolic activation. However, reproducible increases in the mutation frequency (MF) were seen in the presence of S9 activation. In the initial trial, the MF was increased 3.1-fold at 300 µg/mL (MF =201x10^{-6} vs. 66x10^{-6} in the solvent control). The MF was increased ≈2-fold (128x10^{-6} vs. 66x10^{-6} in the control) at a comparable dose in the confirmatory

trial. Although these findings provide convincing evidence of mutagenesis, the response was confined to a single cytotoxic concentration. 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 further note that 1-naphthaleneacetic acid, ethyl ester technical was negative in the mouse micronucleus assay (see MRID No. 43581003). 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-Naphthaleneacetic acid, ethyl ester technical

   Description: Amber liquid
   Lot/batch number: AM 315002
   Purity: 97.75%
   Receipt date: June 10, 1994
   Stability: Not reported
   CAS number: 2122-70-5
   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, 1001 and 2502 µg/mL

Activated conditions: 0.5, 1.0, 5.0, 10, 50, 100, 500, 1001 and 2502 µg/mL

(b)Mutation assay:

(1) Initial Assay:

Nonactivated conditions: Doses of 10-100 µg/mL were assayed; cultures treated with 20, 30, 40, 50 or 60 µg/mL were selected for cloning.

S9-Activated conditions: Doses of 50-500 µg/mL were assayed; cultures treated with 60, 80, 100, 200 or 300 µg/mL were selected for cloning.

(2) Confirmatory Assay:

Nonactivated conditions:	Doses ranging from 20-100 µg/mL were assayed; cultures treated with 40, 50, 60, 70, 80 or 100 µg/mL were selected for cloning.
S9-Activated conditions:	Doses of 60-500 µg/mL were assayed; cultures treated with 70, 80, 100, 200 or 300 µg/ml were selected for cloning.

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. RESULTS:

A. Preliminary Cytotoxicity Assay: The test material was soluble at all assayed doses (0.5-2502 µg/mL) with or without S9 activation. In the nonactivated phase of testing, no cells survived treatment with test doses ≥100 µg/mL. Relative suspension growth (RSG) was 59% at 50 µg/mL. For the remaining nonactivated levels (0.5-10 µg/mL), RSG was ≥100%. In the presence of S9 activation, no cells were recovered at the three highest concentrations (500, 1001 and 2502 µg/mL). Lower concentrations (≤100 µg/mL) were not cytotoxic. Based on these findings, doses of 10 to 100 µg/mL -S9 and 50-500 µg/mL +S9 were selected for testing in the initial mutation assay.

B. Mutation Assays: No appreciable difference between the osmotic pressure of culture medium containing the solvent (DMSO) or 400 µg/mL of the test material was noted. Results from the initial and confirmatory assays were as follows:

Nonactivated conditions: In the initial nonactivated phase of testing, doses ≥80 µg/mL were severely cytotoxic and not cloned. Relative suspension growth (RSG) postexposure to 60 µg/mL was 41%. RSG was dose dependent for the remaining levels, ranging from 48-90% at 50-20 µg/mL, respectively. As the representative data presented in Table 1 indicate, the nonactivated test material did not induce a mutagenic effect. Doses ranging from 20-100 µg/mL were assayed in the confirmatory trial. As shown in Table 1, cytotoxicity was less pronounced and a marked reduction in RSG (≈75%) was confined to the high dose. There was, however, no indication of a mutagenic effect. 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 are presented in Table 2. In the initial trial, no cells survived treatment with levels ≥400 µg/mL; RSG for lower levels was dose dependent (ranging from 18% at 300 µg/mL to 95% at 60 µg/mL). Accordingly, cells exposed to 60, 80, 100, 200 or 300 µg/mL were cloned. The test material caused a dose-related increase in total mutant colonies and the MF at 200 and 300 µg/mL; MFs were 108 and 201×10^{-6}, respectively, at these levels as compared to a solvent control MF of 66×10^{-6}. These values represent increases that were ≈1.6- and 3.1-fold higher than control. It was also noted that total growth (TG) at all levels exceeded 10%. Based on these findings, concentrations ranging from 60-500 µg/mL were processed in the confirmatory trial. The evidence of cytotoxicity and mutagenicity was confirmed in the repeat trial. As further shown in Table 2, marked cytotoxicity was achieved at ≥400 µg/mL but an ≈2-fold increase in the MF with 13% TG was demonstrated at 300 µg/mL. 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 for the high and mid-dosing solutions in both trials were within ±10% of the intended levels.

III. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

A. INVESTIGATORS' CONCLUSIONS: Based on the overall data, the study authors concluded that 1-naphthalene-acetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester 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 mutant, which are thought to represent multiple locus mutations associated with chromosome 11b alterations[1], were induced in the initial but not the confirmatory trial. We note that 1-naphthaleneacetic acid, ethyl ester technical was negative in the mouse micronucleus assay (see MRID No. 43581003). The issue as to whether the test substance has intrinsic clastogenic activity can only be resolved by performance of an in vitro cytogenetic assay.

C. **STUDY DEFICIENCIES**: None.

[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.

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with 1-Naphthaleneacetic Acid, Ethyl Ester 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]	24	143	100	34	--
(Test material)	1%	100[e]	19	161	100	24	--
Dimethyl sulfoxide	1%	100[d]	25	159	100	31	--
(Positive controls)	1%	100[e]	25	172	100	29	--
Positive Control[f]							
Ethylmethane sulfonate	0.25 µL	73[d]	277	126	81	440	14.2
	0.25 µL	72[e]	304	98	57	620	21.4
Test Material							
1-Naphthaleneacetic acid,	60 µg[g]	41[d]	25	154	108	33	<1
ethyl ester technical	80 µg[g]	48[e]	23	147	91	32	1.3
	100 µg	25[h]	17	126	78	27	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 (20, 30, 40 or 50 µg/mL--initial trial or 40, 50, 60 or 70 µg/mL--confirmatory trial) did not induce a mutagenic response.
[h]No cells were recovered from one of the two replicate cultures; with the exception of percent relative growth, results at this dose are based on one culture.

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-Naphthaleneacetic Acid, Ethyl Ester, 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]	44	134	100	100	66	
(Test material)	1%	100[e]	40	123	100	100	66	--
Dimethyl sulfoxide	1%	100[d]	62	135	100	100	92	--
(Positive controls)	1%	100[e]	42	126	100	100	66	--
Positive Control[f]								
7,12-Dimethylbenz(a)-	2.5 µg	83[d]	149	138	76	85	216	2.3
anthracene	2.5 µg	68[e]	131	82	65	44	320	4.9
Test Material								
1-Naphthaleneacetic Acid,	60 µg	95[d]	54	149	111	105	72	1.1
Ethyl Ester Technical	80 µg	95	64	134	100	94	96	1.5
	100 µg	93	47	135	101	93	70	1.1
	200 µg	67	77	142	106	71	108	1.6
	300 µg[g]	18	97	96	72	13	201	3.1
	200 µg[h]	75[e]	44	137	111	83	65	<1
	300 µg[g]	14	70	110	89	13	128	1.9

[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 (≥400 µg/ml--both trials) were severely cytotoxic.
[h]Results for lower doses (70, 80 and 100 µg/mL) did not suggest a mutagenic effect.

Note: Data were extracted from the study report, pp 18, 19, 22 and 23.

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 # 0051960

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: 056008 **TOX. CHEM. NO.**: 589AA

TEST MATERIAL (PURITY): 1-Naphthaleneacetic Acid, Ethyl Ester Technical (97.75%)

SYNONYM(S): None

CITATION: Putman, D.L. and R.R. Young . 1994. Micronucleus Cytogenetic Assay in Mice : Technical 1-Naphthaleneacetic Acid, Ethyl Ester; Microbiological Associates, Inc., Rockville, MD; Report No. G94AU53.122; 36 pages. Study Completion Date: December 12, 1994. MRID: 43581003. Unpublished.

SPONSOR: AMVAC Chemical Corp., Los Angeles, CA

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No: 43581003), groups of five male and five female ICR mice received single intraperitoneal injections of 305, 610 or 1220 mg/kg 1-naphthaleneacetic acid, ethyl ester technical (AM 315002; 97.75%). Based on analytical data, the actual doses used in the study were 162.3, 456.3 and 947.9 mg/kg. 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).

Including the secondary group, death occurred in ≈48% (11/20 ♂ and 8/20 ♀) of the high-dose animals. Lethargy was also noted in the high- and mid-dose males and females. 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-naphthaleneacetic acid, ethyl ester 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-Naphthaleneacetic acid, ethyl ester technical

Description: Amber liquid
Lot/batch number: AM 315002
Purity: 97.75%
Receipt date: June 10, 1994
Stability: Not reported
CAS number: 2122-70-5
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 were analyzed for actual concentration.

2. **Control Materials**:

Negative/Route of administration: None

Vehicle Control /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: 29.1-35.7 g (males); 26.5-33.5 g (females) • Toxicity test Trial I: 30.2–34.3 g (males); 26.7–29.8 g (females) • Toxicity test Trial II: 29.6-33.9 g (males); 25.6-29.9 g (females) • Micronucleus assay: 27.7-36.2 g (males); 23.8-32.1 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)
(b) Toxicity test Trial I: 100, 200, 400 and 800 mg/kg
(5 males and 5 females/dose)
(c) Toxicity test Trial II: 1500 and 3000 mg/kg
(5 males and 5 females/dose)
(d) Micronucleus assay: 305, 610 and 1220 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:

1. **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) and 1000 mg/kg (2 males) died within 24 hours of compound administration. Clinical signs noted prior to death in the two high-dose groups included lethargy at 1000 mg/kg and prostration in both sexes at 5000 mg/kg. Doses ≤100 mg/kg were not toxic.

2. **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 (100, 200, 400 and 800 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. One female administered 800 mg/kg died. The only other sign of clinical toxicity was lethargy in both sexes at levels ≥400 mg/kg. Since the $LD_{50/3}$ could not be determined from these data, the toxicity test was repeated.

3. **Toxicity Test Trial II**: Doses evaluated in the repeat trial were 1500 and 3000 mg/kg administered IP to groups of five male and five female mice per group. All high-dose males and females and one low-dose male succumbed to treatment within 2 hours. Lethargy was reported for both sexes receiving 1500 mg/kg. Based on the combined data from the three preliminary studies, the estimated $LD_{50/3}$, calculated by probit analysis, was 1518 mg/kg. Accordingly, the high dose selected for the micronucleus assay (≈80% of the $LD_{50/3}$) was 1220 mg/kg.

3. **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 53.2, 74.8 and 77.7%, respectively. Based on these data, the actual doses used in the study were 162.3, 456.3 and 947.9 mg/kg.

(b) **Animal observations**: In the high dose group, 11 of 20 males and 8 of 20 females died prior to the scheduled sacrifice and were replaced with animals from the secondary group. Due to the increased incidence of mortality in the high-dose group, only 8 animals (4 males and 4 females) were available for the 48-hour bone marrow harvest and only 3 females were available from the 72-hour harvest. No unscheduled deaths occurred in the males or females of the remaining treatment groups. Other clinical signs of compound toxicity included lethargy in the mid- and high-dose groups. All remaining animals appeared normal throughout the course of study.

(c) **Bone marrow analysis**: Summarized results from the micronucleus assay conducted with the test substance administered IP to male and female mice are presented in Study Report Table 2, p 17 (see Attachment). As shown, a moderate reduction (≈43% 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. Slight reductions (31 or 21%) were also noted in the high-dose females at 48 and 72 hours postexposure, respectively. Although reduced PCE:NCE ratios were observed in the males and females receiving the intermediate dose, the responses were marginal and not clearly indicative of an adverse effect on erythropoiesis. 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-naphthalene-acetic acid, ethyl ester technical was negative in the mouse micronucleus assay.

III. **REVIEWERS' DISCUSSION/CONCLUSIONS**: We agree with the study authors' assessment that 1-naphthaleneacetic acid, ethyl ester technical was neither clastogenic nor aneugenic in this in vivo assay. The evidence of overt compound toxicity in conjunction with the depressed PCE:NCE ratios seen in the high-dose group 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-naphthaleneacetic acid, ethyl ester 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.

E. STUDY DEFICIENCIES: Although actual concentrations for all dosing suspensions were considerably lower than the target levels, this deficiency did not compromise the integrity of the study. The data clearly showed that the high dose of the test material was overtly toxic to the animals and cytotoxic to the target organ. Therefore, the lack of a positive response was not due to the inability of the test material to interact with genetic material.

ATTACHEMENT[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-Naphthalene acetic acid, ethyl ester	M	24	5	0.57	0.0 ± 0.00	0/5000
		48	5	0.55	0.4 ± 0.55	2/5000
		72	5	0.61	0.4 ± 0.89	2/5000
305 mg/kg	F	24	5	0.53	0.4 ± 0.55	2/5000
		48	5	0.60	0.6 ± 0.89	3/5000
		72	5	0.67	0.4 ± 0.89	2/5000
	M	24	5	0.53	0.4 ± 0.89	2/5000
		48	5	0.44	0.2 ± 0.45	1/5000
610 mg/kg		72	5	0.66	0.6 ± 0.55	3/5000
	F	24	5	0.48	0.2 ± 0.45	1/5000
		48	5	0.50	0.6 ± 0.55	3/5000
		72	5	0.76	0.2 ± 0.45	1/5000
	M	24	5	0.30	0.2 ± 0.45	1/5000
		48	4	0.41	0.3 ± 0.50	1/5000
		72	0			
1220 mg/kg	F	24	5	0.47	0.4 ± 0.55	2/5000
		48	5	0.42	0.5 ± 1.00	2/5000
		72	5	0.55	1.0 ± 1.73	3/5000
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 43581003, Table 2, page 17.

DATA EVALUATION RECORD

1-NAPHTHALENEACETIC ACID, ETHYL ESTER/056008

STUDY TYPE: METABOLISM AND PHARMACOKINETICS - RAT
[OPPTS: 870.7485 (§85-1)]
MRID 43961701

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. 03-22

Primary Reviewer: William J. Spangler, Ph.D..	Signature: Date:	Robert H. Ross for W. J. Spangler SEP 10 2003
Secondary Reviewers: Robert A. Young, Ph.D., D.A.B.T.	Signature: Date:	Robert A. Young SEP 10 2003
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross SEP 10 2003
Quality Assurance: LeeAnn Wilson, M.S.	Signature: Date:	L. A. Wilson SEP 10 2003

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

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:** A. Khasawinah
Reregistration Branch 4, Health Effects Division (7509C) **Date** Sept. 22, 2003
EPA Secondary Reviewer: William Dykstra, Ph.D. **Signature:** W. Dykstra
Reregistration Branch 4, Health Effects Division (7509C) **Date** 9/25/03
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/30/03

TXR#: 0051960

DATA EVALUATION RECORD

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

PC CODE: 056008 **DP BARCODE:** D229632
SUBMISSION NO.:S511187

TEST MATERIAL (PURITY): 1-Naphthaleneacetic acid, ethyl ester (99.3%)

SYNONYMS: Not available

CITATION: McCorquodale, G.Y. and M.S. Prout. 1996. The metabolism of [^{14}C]-1-naphthaleneacetic acid, ethyl ester in the rat following oral administration. Report No. 12149, Iveresk Research International, Tranent, EH33 2NE, Scotland. 20 March 1996. Study No. IRI 154702. Unpublished, 124 pages. MRID 43961701.

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

EXECUTIVE SUMMARY: In a study (MRID 43961701) conducted to examine the metabolism and disposition of 1-naphthaleneacetic acid, ethyl ester, male and female Sprague-Dawley rats were given 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-naphthaleneacetic acid, ethyl ester (Batch No. CSL-94-516-33-25, 99.3% radiochemical purity, specific acttivity 56.2 mCi/mmol), and nonlabeled test article (Batch No. GAB 69-34-02, chemical purity not available). Excretion, tissue distribution, pharmacokinetic parameters, 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 98.6-101.8%. 1-Naphthaleneacetic acid, ethyl ester was readily absorbed and excreted within 36 - 48 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-naphthaleneacetic acid, ethyl ester, urinary excretion accounted for 67.6-85.3% of the administered radioactivity. Urinary excretion was unaffected following a single 100 mg/kg bw dose with 61.8-78% of the administered radioactivity excreted in urine. Excretion via the feces accounted for the remainder of the administered radioactivity excreted by all treatment groups (12.3-35.2%). Excretory patterns did not exhibit gender-related variability for the low dose

groups although a minor difference was observed at the high dose. Excretion patterns of the high-dose group reflected delayed absorption. Because tissue burdens were very low at termination, neither 1-naphthaleneacetic acid, ethyl ester nor its metabolites appear to undergo significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC, TLC and most were identified using HPLC, GC/MS, and HPLC/MS in conjunction with known standards. The major pathway of metabolism involved ester cleavage followed by glycine and glucuronide conjugation at the low and low repeat doses. At the high dose, glucuronide conjugation appeared to play a more important role following ester cleavage. Parent compound was detected at low concentrations (0.5-4.7% 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-naphthaleneacetic acid, ethyl ester, affirmed the metabolism pathway proposed by the investigators.

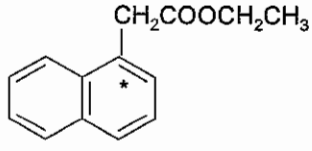
This metabolism study (MRID 43961701) is **Acceptable/Guideline** and satisfies the requirements for a Tier 1 Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)] to determine the routes and rate of excretion and to identify excreted metabolites in male and female rats. The study was properly designed, conducted and reported.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	ring labeled [^{14}C]-1-naphthaleneacetic acid, ethyl ester
Radiochemical purity	99.3%
Chemical purity	Not available
Specific Activity	56.2 mCi/mmol (9.705 MBq/mg)
Batch #:	CSL-99-516-33-25
Non-radiolabelled test material:	1-naphthaleneacetic acid, ethyl ester
Description:	White powder
Batch #:	GAB 69-34-02
Purity:	Not reported
Contaminants:	Not reported
CAS # of TGAI:	Not reported
Structure:	

2. **Vehicle:** The vehicle for labeled and unlabeled 1-naphthaleneacetic acid, ethyl ester was corn oil. The dosing solutions were prepared by drying the measured dose under nitrogen to remove the acetonitrile solvent then added to a weighed volume of corn oil. The test material was dissolved using stirring and sonication.

3. **Test animals:**

Species:	Rat (male and female)	
Strain:	Sprague-Dawley	
Age/weight at study initiation:	7-8 weeks; 190-220 g (males), 170-190 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-naphthaleneacetic acid, ethyl ester in a weighed portion of corn oil and dissolving with stirring and sonication. Solvent (acetonitrile) was removed from the labeled test article under nitrogen prior to mixing with corn oil. 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-naphthaleneacetic acid, ethyl ester in a weighed portion of corn oil and dissolving with stirring and sonication. The high-dose suspension (100 mg/kg bw) was prepared by dissolving appropriate amounts of [^{14}C]-labeled and unlabeled 1-naphthaleneacetic acid, ethyl ester in acetonitrile. An appropriate amount of this solution was dried in a vial under nitrogen and the proper weighed volume of corn oil added and dissolved 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 after dosing. The stability of the dosing suspensions was determined by measuring the radioactivity over a 28 day period. The radioactive purity was determined by TLC to be 98.5% after 28 days.

B. STUDY DESIGN AND METHODS:

1. **In life dates** - Start: 08/11/94 End: 08/08/95

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-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester followed by single dose of [^{14}C]-1-naphthaleneacetic acid, ethyl ester 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. 20-22, MRID 43961701.

a. **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.

b. **Analytical techniques and metabolite characterization 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 were collected at each fecal collection interval and retained at room temperature for subsequent analysis.

Tissue/organs: The following organs/tissues were collected from rats in Groups 2, 3, and 6 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.

3. **Analytical techniques:**

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 Groups 1, 2, and 3 males and females were directly injected for HPLC analysis without further treatment. Pooled 0-48 hour feces from Groups 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-naphthaleneacetic acid, ethyl ester using the following solvent systems: toluene (100%) and hexane:ethyl acetate (5:1, 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. The columns, mobile phases and operating conditions were adequately described in the study report.

Gas Chromatography Mas Spectrometry (GC-MS: GC-MS was performed as necessary using a HP5890 series II gas chromatograph coupled to a VG Masslab Trio-IS mas spectrometer. Reference standards and selected HPLC fractions were analyzed by GC-MS using the respective *tert*-butyldimethylsilyl derivatives.

4. **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 98.6-101.8%. A general accounting of recovered radioactivity following dosing with [^{14}C]-1-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester

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	0.12±0.03	0.12±0.04	0.13±0.03	0.07±0.02	0.10±0.05	0.23±0.14
Urine	75.0±5.05	67.6±5.38	82.1±6.06	85.3±4.10	61.8±10.9	78.0±6.76
Feces	20.6±5.00	28.4±5.14	16.5±4.94	12.3±2.31	35.2±11.8	17.3±5.50
Cage wash	3.12±2.27	2.45±1.45	3.02±2.08	3.28±1.90	2.30±1.14	5.19±2.50
Total	98.8±0.58	98.6±0.59	101.8±1.16	100.1±1.52	99.4±0.77	100.7±1.32

Data obtained from Tables 1, 4, and 7, pp 41, 44, and 47, MRID 43961701.

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 on radioactivity recovered in these matrices, absorption following a single low dose or single high dose, respectively, was approximately 70.2-78.2% and 64.2-83.4%. Results from the 14-day repetitive dose study (85.3-88.7%) exhibited an excretion profile similar to that of the single low-dose or single high-dose groups and therefore, similar implied absorption. Absorption rates were similar in both males and females.

Excretion: Time-course excretion data are summarized in Tables 3 and 4. The data indicated that most of the administered radioactive dose is excreted in the urine; 67.6-85.3% for the low dose groups and 61.8-78% for the high-dose group. Most (95.5-99.1%) of the urinary excretion of administered radioactivity occurred within 24 hours for the low-dose rats and within 36 hours for high-dose rats. Fecal excretion of the absorbed dose is relatively rapid and essentially complete within 48 hours for all dose groups (97.7-99.6% of excreted radioactivity). There were no biologically relevant gender-related quantitative differences in low-dose excretion profiles. For the high-dose group, females excreted greater amounts (78%) in the urine than did males (61.8%). 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). Urinary excretion in the repetitive low-dose group was significantly higher (82.1-85.3% of administered) than for single low-dose (67.6-75%) and high-dose animals (61.8-78%), whereas fecal excretion in the repetitive low-dose group animals was significantly lower (12.3-16.5% of administered) than low-dose (20.6-28.4%) and high-dose animals (17.3-35.2%). This is indicative of induction of metabolic enzymes, involved in urinary excretion, by repeated low doses of the parent compound. Biliary excretion was not assessed.

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

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]	30.3	37.5	25.3	24.1	24.9	18.0
6-12	34.8	26.7	40.2	50.1	15.0	19.0
12-24	7.9	2.8	12.9	9.5	19.6	32.3
24-36	1.4	0.3	3.1	1.1	1.7	6.3
36-48	0.4	0.2	0.3	0.3	0.4	1.9
48-72	0.1	0.0	0.2	0.1	0.1	0.2
72-96	0.1	0.1	0.0	0.0	0.0	0.1
96-120	0.0	0.0	0.1	0.1	0.0	0.1
120-144	0.0	0.0	0.0	0.0	0.0	0.1
144-168	0.0	0.0	0.0	0.0	0.1	0.0
Total	75.0	67.6	82.1	85.3	61.8	78.0

Data taken from Tables 1, 4, and 7, pp. 41, 44, and 47, MRID 43961701.
[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-naphthaleneacetacetic acid, ethyl ester

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]	8.5	19.0	1.4	1.8	12.2	0.4
12-24	9.0	8.9	8.7	6.2	15.1	9.0
24-36	2.0	0.3	5.0	3.7	4.7	4.9
36-48	0.9	0.1	1.1	0.4	3.0	2.6
48-72	0.2	0.1	0.2	0.1	0.1	0.2
72-96	0.0	0.0	0.1	0.1	0.0	0.0
96-120	0.0	0.0	0.0	0.0	0.0	0.1
120-144	0.0	0.0	0.0	0.0	0.1	0.0
144-168	0.0	0.0	0.0	0.0	0.0	0.0
Total	20.6	28.4	16.5	12.3	35.2	17.3

Data taken from Table 1, 4, and 7, pp. 41, 44, and 47, MRID 43961701.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

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

B. TISSUE DISTRIBUTION: The distribution of the administered radioactivity in 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, and residual carcass. Mean tissue burdens at termination accounted for only 0.12% of the administered radioactivity in both males and females. Although accumulation in blood was not significant, radioactivity was higher in whole blood than in plasma. For repeat dose animals the highest tissue burdens were found in the kidneys, liver, and residual carcass at concentrations similar to single low-dose animals. At termination, mean tissue burdens accounted for only 0.13 and 0.07% of the

administered radioactivity in males and females, respectively. Similar to the low-dose animals, radioactive residues were generally higher in females than males. 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 from the low- to high-dose animals. Again, the highest tissue burdens were found in the kidneys, liver, and residual carcass but were generally higher in females (0.23% of administered radioactivity) than in males (0.10% of administered dose). The highest tissue burdens for the high-dose group were found in the renal fat of females (1.51 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.

With the possible exception of the renal fat of high-dose females, 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 (0.07-0.23%). Although no significant overall accumulation occurred in blood, whole blood accumulated more radioactivity than plasma indicating the site of accumulation was the red blood cells.

TABLE 5. Total radioactive residue (µg/g) in organs and tissues in rats168 hrs following oral administration of [14C]-1-naphthaleneacetic acid, ethyl ester

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	0.0001±0.0000	0.0005±0.0001	0.0002±0.0000	0.04±0.04	0.12±0.12
Carcass	0.0012±0.0002	0.0014±0.0006	0.0015±0.003	0.0008±0.0002	0.09±0.05	0.23±0.16
Bone	0.0002±0.0000	0.0002±0.0000	0.0003±0.0001	0.0002±0.0000	0.02±0.01	0.05±0.02
Brain	0.0000±0.0000	0.0000±0.0000	0.0001±0.0000	0.0001±0.0000	0.00±0.00	0.02±0.01
Renal Fat	0.0007±0.0004	0.0008±0.0005	0.0008±0.0005	0.0005±0.0002	0.16±0.19	1.51±1.94
Heart	0.0001±0.0000	0.0002±0.0001	0.0003±0.0001	0.0002±0.0001	0.02±0.01	0.04±0.02
Kidneys	0.0021±0.004	0.0025±0.0001	0.0042±0.0003	0.0036±0.0006	0.16±0.04	0.29±0.03
Liver	0.0018±0.0004	0.0017±0.004	0.0035±0.0008	0.0019±0.0007	0.23±0.04	0.32±0.09
Lungs	0.0003±0.0001	0.0003±0.0001	0.0005±0.0002	0.0004±0.0001	0.03±0.01	0.06±0.01
Muscle	0.0002±0.0000	0.0001±0.0000	0.0001±0.0000	0.0001±0.0000	0.01±0.01	0.02±0.01
Ovaries	NA	0.0004±0.0003	NA	0.0003±0.0001	NA	0.36±0.37
Plasma	0.0003±0.0000	0.0002±0.0001	0.0004±0.0001	0.0002±0.0000	0.02±0.01	0.03±0.00
Spleen	0.0002±0.0000	0.0002±0.0000	0.0005±0.0002	0.0004±0.0001	0.02±0.01	0.04±0.01
Testes	0.0001±0.0000	NA	0.0001±0.0000	NA	0.05±0.06	NA
Whole Blood	0.0008±0.0002	0.0007±0.0001	0.0017±0.0008	0.0011±0.0004	0.06±0.02	0.11±0.04

Data obtained from Tables 2, 5, and 8, pp. 42, 45, and 48, MRID 43961701.

C. METABOLITE CHARACTERIZATION STUDIES:

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

NAA	Naphthaleneacetic acid
NAA-Gluc	Naphthaleneacetic acid glucuronide conjugate
NAA-Glyc	Naphthaleneacetic acid glycine conjugate
HO-NAA	Hydroxy-naphthaleneacetic acid
NAA-Et	1-Naphthaleneacetic acid, ethyl ester

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-naphthaleneacetic acid, ethyl ester included seven metabolite fractions, including an unidentified polar fraction and three isomers of HO-NAA (Table 6.). All were recovered at ≥0.5% of the administered dose. The major metabolites detected were the glucuronide and glycine conjugates of naphthaleneacetic acid (3.4-20.4% and 16.8-57.2% of the administered dose, respectively) with the glycine conjugate being identified as the predominate metabolite, especially in low-dose animals. Other metabolites detected in urine were HO-NAA isomers (16.0-24.7% of administered), polar fraction (0.5-1.7% of administered), and NAA (0.5-10.2% of administered). No biologically significant qualitative or quantitative differences were noted for males or females in any dose group, although the total recovered radioactivity for the repetitive low-dose group was the highest of the three dose groups. Parent compound was not detected in urine.

TABLE 6. Metabolite profile in urine of rats following oral dosing with [^{14}C]-1-naphthaleneacetic acid, ethyl ester expressed as % of recovered 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
Polar	**4.3**	1.1 (0.8)	0.8 (0.5)	1.2 (1.0)	0.6 (0.5)	2.8 (1.7)	0.8 (0.6)
HO-NAA	**6.3**	11.7 (8.8)	9.3 (6.3)	17.4 (14.3)	12.2 (10.4)	10.5 (6.5)	9.1 (7.1)
HO-NAA	**7.0**	4.9 (3.7)	2.6 (1.8)	6.7 (5.5)	4.0 (3.4)	9.8 (6.1)	6.8 (5.3)
NAA-Gluc	**9.0**	9.5 (7.1)	8.6 (5.8)	8.3 (6.8)	4.0 (3.4)	26.6 (16.4)	26.1 (20.4)
NAA-Glyc	**9.8**	62.0 (46.5)	64.4 (43.5)	54.5 (44.7)	67.0 (57.2)	27.2 (16.8)	33.0 (25.7)
HO-NAA	**10.3**	5.0 (3.8)	9.3 (6.3)	6.0 (4.9)	7.4 (6.3)	13.3 (8.2)	4.6 (3.6)
NAA	**12.5**	1.9 (1.4)	0.7 (0.5)	2.4 (2.0)	0.8 (0.7)	5.8 (3.6)	13.1(10.2)
Unaccounted[b]		2.9	2.9	2.9	3.4		
Identified metabolites as % of recovered		96.1 (72.1)	95.7 (64.7)	96.5 (79.2)	96.0 (81.9)	96.0 (59.3)	93.5 (72.9)
% Dose excreted in urine		75.0	67.6	82.1	85.3	61.8	78.0
Total[c]		96.1	95.7	96.5	96.0	96.0	93.5

Data from Table 10, p. 50, MRID 43961701.
[a]RT = HPLC retention time in minutes
[b] Unaccounted = Total urinary recovery (% of administered dose) - Total Identified/accounted
[c] Total = % of total urinary radioactivity (Total identified/accounted ÷ Total urinary recovery)

Feces: The fecal metabolite profile for rats given [^{14}C]-1-naphthaleneacetic acid, ethyl ester is given in Table 7. Six fractions including an unidentified polar fraction and one isomer of HO-NAA were observed in the fecal extracts. The major metabolites isolated from feces were the glucuronide and glycine conjugates of naphthalene acetic acid (1.5-7.8 and 1.0-5.9% of administered, respectively). Other metabolites identified were HO-NAA (0.0-1.9%), NAA (1.7-4.4%) and a polar fraction (2.0-11.4% of administered). Parent compound was not the prevalent component extracted from feces (0.5-4.7% of administered) and total fecal metabolites accounted for 12.3-35.2% of the administered dose. Similar to urine metabolite profiles, there were no significant qualitative differences between males and female but marked quantitative differences were observed. Metabolism of 1-naphthaleneacetic acid, ethyl ester was primarily by ester cleavage followed by glucuronide or glycine conjugation of the resultant naphthalene acetic acid moiety.

TABLE 7. Metabolite profile in feces of rats following oral dosing with [^{14}C]-1-naphthaleneacetic acid, ethyl ester expressed as % of recovered 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
Polar	3.8	31.1 (6.4)	23.8 (6.8)	34.2 (5.6)	16.2 (2.0)	32.4 (11.4)	12.1 (2.1)
HO-NAA	8.5	8.5 (1.8)	6.7 (1.9)	4.0 (0.7)	6.9 (0.8)	– (0.0)	10.8 (1.9)
NAA-Gluc	9.0	7.7 (1.6)	27.4 (7.8)	11.1 (1.5)	25.4 (3.1)	18.5 (6.5)	12.8 (2.2)
NAA-Glyc	9.8	7.0 (1.4)	6.7 (1.9)	9.2 (1.5)	8.3 (1.0)	16.9 (5.9)	10.0 (1.7)
NAA	12.5	8.7 (1.8)	11.4 (3.2)	20.6 (3.4)	14.2 (1.7)	9.8 (3.4)	25.5 (4.4)
NAA-Et	17.8	22.9 (4.7)	15.4 (4.4)	3.2 (0.5)	4.4 (0.5)	12.5 (4.4)	6.2 (1.1)
Unaccounted[b]		2.9	2.4	3.3	3.2	3.6	3.9
Identified metabolites as % of recovered		85.9 (17.7)	91.4 (26.0)	82.3 (13.2)	75.4 (9.1)	90.1 (31.6)	77.4 (13.4)
% Dose excreted in feces		20.6	28.4	16.5	12.3	35.2	17.3
Total[c]		85.9	91.5	80.0	74.0	89.8	77.5

Data from Table 11, p. 51, MRID 43961701
[a]RT = HPLC retention time in minutes
[b] Unaccounted = Total recovery in feces(% of administered dose) - Total Identified/accounted
[c] Total = % of total radioactivity in feces (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 98.6-101.8%. Based upon urinary excretion, ~68-75% of the single low dose and 82.1-85.3% of the repeated low dose was absorbed. Following oral administration, 1-naphthaleneacetic acid, ethyl ester was rapidly absorbed and excretion was nearly complete in 24 hours at the low-dose and 36 hours at the high-dose. At sacrifice, tissue residues of the administered radioactivity were low (0.1-0.23%) with the highest concentrations found in the kidneys, liver, and residual carcass. At termination there were no signs of bioaccumulation in tissues and organs with the possible exception of renal fat of high-dose females. Urine was the major route of excretion (61.8-85.3% of administered

dose) with >90% of the urinary excretion occurring within 24 hours at the single-low and repeat-low doses and within 36 hours at the high-dose. Feces accounted for 12.5-35.2% of the administered dose. However only a small percentage of the dose (0.5-4.7%) was excreted as parent compound. Excretion data did not indicate biologically important, gender-related quantitative differences in low-dose excretion profiles but did suggest minor gender differences and 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 (~44-57% of administered). The glucuronide conjugate was also a major metabolite at the low doses (3.4-7.1% of administered) but was present in comparatively higher concentrations for high-dose animals (16.4-20.4% of administered) compared to the glycine conjugate of naphthalene acetic acid (16.8-25.7% of the administered radioactivity). Other minor metabolites identified in urine were a polar fraction, HO-NAA isomers, and NAA (0.5-1.7, 14.4-24.7, and 0.5-10.2% of administered, respectively). For feces, the major metabolites detected were the polar fraction and the glycine and glucuronide conjugates of naphthalene acetic acid (2.0-11.4, 1.0-5.9, and 1.5-7.8% of administered radioactivity, respectively). Parent compound (NAA-Et) was detected at low concentrations (0.0.5-4.7% of administered) only in feces. The study authors proposed the metabolic scheme depicted in Figure 1 for the metabolism of 1-naphthaleneacetic acid, ethyl ester. The major pathway involved ester cleavage followed by glycine and glucuronide conjugation with the resultant naphthaleneacetic acid moiety.

B. <u>REVIEWER COMMENTS</u>: This study (MRID 43961701) was conducted to examine the metabolism and disposition of 1-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester (Batch No. CSL-94-516-33-25; 99.3% radiochemical purity; chemical purity not stated) and nonlabeled test article (Batch No. GAB 69-34-02; 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-naphthaleneacetic acid, ethyl ester 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-naphthaleneacetic acid, ethyl ester is readily absorbed and rapidly excreted within 36-48 hours following a single or repeated oral dose of 1 mg/kg or a single dose at 100 mg/kg bw. Following single low (1 mg/kg) doses or multiple oral low doses (1 mg/kg) of 1-naphthaleneacetic acid, ethyl ester, urinary excretion accounted for 75-82.1% and 67.6-85.3.0% of the administered radioactivity in males and females, respectively. Following a single high-dose (100 mg/kg bw) of 1-naphthaleneacetic acid, ethyl ester, urinary excretion accounted for 61.8% and 78.0% of the administered radioactivity in males and females, respectively. When urinary excretion for single-low and single-high doses (67.6-75% and 61.8-78% of administered, respectively) is compared to repeated low-doses (82.1-85.3% of administered), it appears that multiple dosing induced higher levels of the enzymes involved in the urinary excretion process. Excretion via the feces accounted for the remainder of the excreted radioactivity in all treatment groups (12.3-35.2%). Although >20% of the administered dose was excreted in feces of some dose groups, it was not

considered necessary to perform intravenous administration or perform a bile cannulation study because only 0.5-4.7% 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-naphthaleneacetic acid, ethyl ester and/or its metabolites do not appear to undergo any significant sequestration in most tissues.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC, GC/MS 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 (43.5-57.2% of administered). The glucuronide conjugate was also a major metabolite at the low doses (3.4-7.1% of administered) but was present in comparatively higher concentrations for high-dose animals (16.4-20.4% of administered). For feces, the major metabolites detected were the polar fraction (2.0-11.4% of administered) and the glucuronide and glycine conjugates of naphthaleneacetic acid (1.5-7.8% and 1.5-5.9% of the administered radioactivity, respectively). Parent compound (NAA-Et) was detected at low concentrations (0.5-4.7% of administered) only in feces. The study authors proposed the metabolic scheme depicted in Figure 1 for the metabolism of 1-naphthaleneacetic acid, ethyl ester. The major pathway involved ester cleavage followed by glycine and glucuronide conjugation at the low dose. At the high dose glucuronide conjugation appeared to play a more important role following ester 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 43961701) 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.

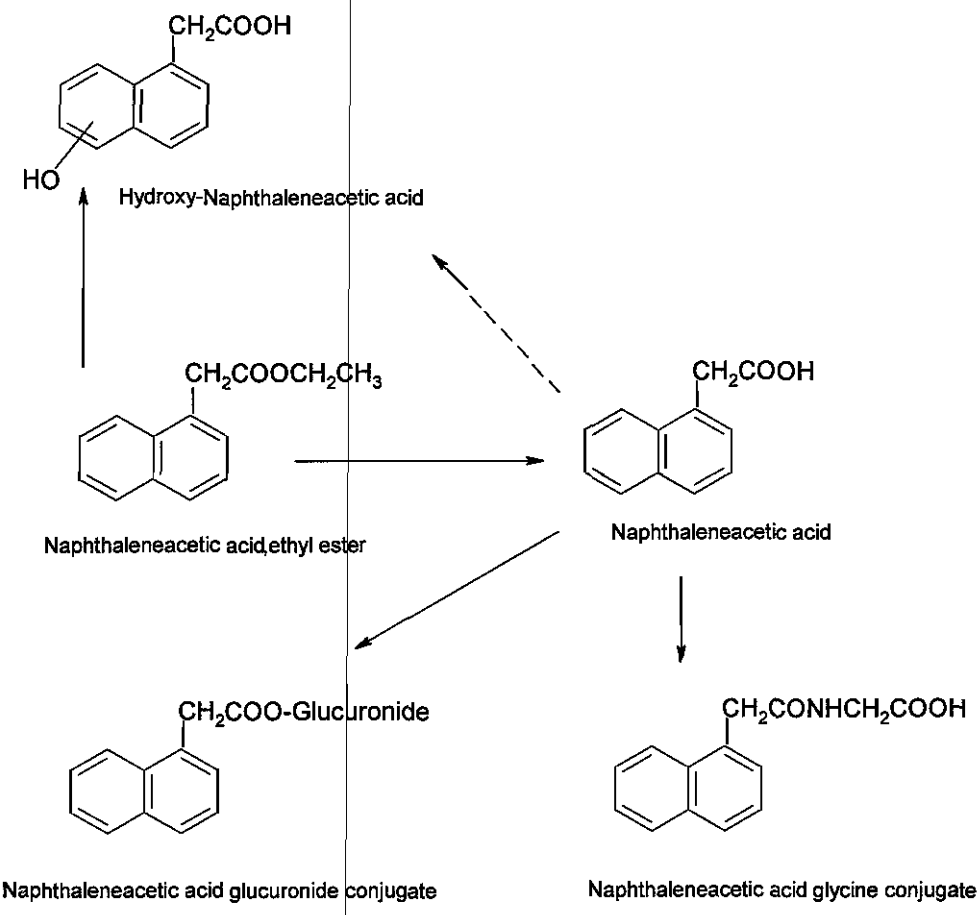


Figure 1. Proposed Metabolic Pathway of 1-Naphthaleneacetic Acid, Ethyl Ester in the Rat

13544

R099679

Chemical:	**Ethyl 1-naphthaleneacetate**
PC Code:	**056008**
HED File Code	**13000 Tox Reviews**
Memo Date:	**01/29/2004**
File ID:	**TX0051960**
Accession Number:	**412-04-0139**

HED Records Reference Center
06/03/2004