UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: January 7, 1980

SUBJECT: EPA Reg.#264-GGA; PP#0F2277; petition proposing establishment of tolerances for residues of ethyl-naphthaleneacetic acid in or on apples, pears and olives. CASWELL#589

FROM: William Dykstra WSW 1/7/80 WSW
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TO: Robert Taylor & Residue Chemistry Branch
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THRU: Dr. Adrian Gross, Chief William M. Britt for McAdam Gross
Toxicology Branch (TS-769)

Petitioner: Union Carbide
Agricultural Products Co., Inc.
Amber, Pa. 19002

Recommendations

1) The requested tolerances are not toxicologically upported.

2) The following toxicity studies are required:

   a) A rat metabolism study with (C^{14}) ethyl-naphthalene-acetic acid which demonstrated that 1-Naphthalene-acetic acid is primarily formed.

   b) Repeat of the 6-month dog feeding study which did not show a NOEL at the low-dose of 50 mg/kg/day NAA.

3) Teratology study in a second species is required to be submitted within a reasonable period of time.

4) The Toxicology studies submitted are acceptable as Core-Minimum Data.

Section F - Proposed amendment to CFR 180.155, residue tolerances for 1-Naphthaleneacetic acid.

This petition is intended to amended the currently established residue tolerance (CFR 180.155) for 1-Naphthaleneacetic acid to permit application of either 1-Naphthaleneacetic acid or the ethyl ester of 1-Naphthaleneacetic acid to apples, pears and olives.

The petitioner proposes that the wording of CFR 180.155 which established residue tolerances for 1-Naphthaleneacetic acid be amended to read as follows:

- 1.0 ppm in or on apples and pears from the application of 1-Naphthaleneacetic acid or the ethyl ester of 1-Naphthaleneacetic acid.

- 0.10 ppm in or on olives from the application of 1-Naphthaleneacetic acid or the ethyl ester of 1-Naphthaleneacetic acid.

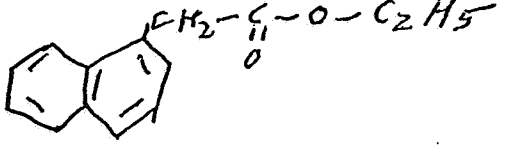
A. Chemical Identification

1. Chemical Name: Ethyl-1-naphthaleneacetic ACID

2. Synonyms: All2, ethyl alpha naphthaleneacetic ACID

3. Purity: 97% pure

4. Structure:



B. Amount, Frequency and Time of Application for Amchem Sprout Inhibitor All2.

1. Amount - Add 8.0 gallons of Amchem Sprout Inhibitor All2 to 92.0 gallons of water. Five to 20 gallons of white exterior latex paint may be substituted for an equal volume of water. The number of trees treated will vary with size and types of application (basal sprouts, scaffold limbs and/or primary cuts). One gallon of spray mixture will generally treat 5.0 to 100 trees.

2. Frequency - Only one application per season is recommended to control basal sprouts, pruning cut sprouts or sprouts originating from scaffold limbs.

3. Time of Application - The time of application is dependent upon the type of treatment.

   a) Basal Sprouts - Treat during the dormant period prior to resprouting or when new shoots are 6-12 inches long. On bearing trees do not treat suckers during bud swell, bloom or fruit set. This period is from the start of growth to 4 weeks after petal fall.

   b) Scaffold Limbs, Water Sprouts and Pruning Cuts - Pruning and treatment should be conducted during the dormant season before bud activity begins.

C. Formulation - Amchem Sprout Inhibitor A112

Ingredient	Percent Weight
Ethyl-1-Naphthaleneacetic ACID	15.56
[redacted]	
	100.00

Inerts cleared under 180.1001 (c)(d)(e).

Review

1. Previously submitted toxicity data.

a) PP#1E1094

°Rat Oral LD_{50} of 1-NAA = 1 gm/kg

°Rat I.P. LD_{50} of 1-NAA = 100 mg/kg

°3-generation mouse with methylester of 1-NAA = 600 ppm (highest dose). Life-span of mice was unaffected at this same level.

°90-Day Rat, 1-NAA: NOEL = 100 mg/kg

°90-Day Dog, 1-NAA: NOEL = 10 mg/kg

°Subcutaneous injections of 1-NAA to 2 mg/kg daily into mice of 7 to 60 days of age produced no effects on growth, body length or development.

°2-Year Rat Feeding Study: NOEL = 2500 ppm; methyl ester of 1-NAA

NOTE: It is noted that in some studies, the methyl ester of 1-NAA was used instead of the salt. This is acceptable for two main reasons:

1) 1-NAA is the principle metabolite of the methyl ester.

2) Even though the exact amount of the 1-NAA arising from the methyl ester is not known, the toxicity of both compounds are similarly low.

b) PP#7E1956

°Rat metabolism of 1-NAA (C^{14}), J. Agr. Food. Chem. 14, 532, 1966.

Protcol: Single oral doses of 0.1, 1.0, 100 and 250 mg of 1-NAA (C^{14}) were given to male rats. Radioactivity was monitored daily in the urine and feces and every 2 hours in the bile. Results: The total radio-label excreted in 3 days was: In urine - 71-91% In feces - 3-20% In bile - 4-21%, 2-6 hours

The two major metabolites (70-93%) in urine was naphthaceturic and naphthacetyl-glucosiduronic acids.

For the lower doses, the excretion in urine and feces in 24 hours was approximately 90%.

Conclusion: 1-NAA and metabolites are well excreted in the urine and feces.

2. New Toxicity DATA Submitted.

A. Mutagenicity and Teratology

1. Yeast (Sacchromyces cerevisiae) strain D-7 Mitotic Crossing Over Assay on -Naphthalene Acetic Acid (Pharmakon Laboratories, 7/17/78).

Test Material: 1-Naphthalene Acetic Acid, Lot No. GN-2095

The purpose of the study was to evaluate the ability of 1-Naphthaleneacetic acid (NAA) to induce mitotic crossing over in the heteroallalic ade 2-40/ade 2-119 diploid strain D-7 of Sacchromyces cerevisiae.

Method: NAA was soluble in phosphate buffer at pH 7.0. 10% DMSO was added to the 10^{-2} molar concentration to increase solubility. A simultaneous control of 10% DMSO in phosphate buffer was included. The preliminary toxicity screen showed no toxicity at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-4}, 10^{-5} and 10^{-6} M.
These same concentrations of NAA were used in the assay.

Results: The results for NAA were negative in the yeast strain D-7 mitotic crossing over assay.

Conclusion: NAA is non-mutagenic under conditions of this assay.

Classification: Core-Minimum Data

2. Yeast (Sacchromyces cerevisiae) Strain D-7 reverse mutation assay on 1-Naphthaleneacetic acid (Pharmakon Laboratories, 7/17/78).

Test Material: 1-Naphthaleneacetic acid; Lot No. GN-2095

The purpose of the study is to evaluate the ability of 1-Naphthaleneacetic acid (NAA) to induce reverse mutation in the homoallelic ilv I-92/ilv I-92 diplorid strain D-7 of Sacchromyces cerevisiae.

Method: NAA was soluble in phosphate buffer at pH 7.0, 10% DMSO was added to the 10^{-2} molar concentration to increase solubility. A simultaneous control of 10% DMSO in phosphate buffer was included. The preliminary toxicity screen showed no toxicity at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M. These same concentrations of NAA were used in the assay.

Results: The results for NAA were negative in the yeast strain D-7 reverse mutation assay.

Conclusion: NAA is not mutagenic in the this assay.

Classification: Core-Minimum Data

3. Yeast (Sacchromyces cerevisiae) strain D-7 mitotic gene conversion assay on 1-Naphthaleneacetic acid (Pharmakon Laboratories, 7/17/78).

Test Material: 1-Naphthaleneacetic acid; Lot No. N-2095

The purpose of the study was to evaluate the ability of 1-Naphthaleneacetic acid (NAA) to induce mitotic gene conversion in the heteroalleleic diploid trp 5-12/trp 5-27 D-7 strain of Sacchromyces cerevisiae.

Method: NAA was soluble in phosphate buffer at pH 7.0. 10% was added to the 10^{-2} molar (1.86 mg/ml), 1.0 mg/ml, and 3.9 mg/ml concentrations to increase solubility. A simultaneous control of 104. DMSO in phosphate buffer was included. The preliminary toxicity screen showed no toxicity at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M. These concentrations were used in the assay. The assay was repeated at the 10^{-2} M (1.86 mg/ml), 1.0 mg/ml and 3.9 mg/ml concentrations in an attempt to duplicate the initial results.

Results: The results for NAA were negative in the Yeast Strain D-7 mitotic conversion gene assay.

Conclusion: NAA is not mutagenic in this test system.

Classification: Core-Minimum Data

4. Escherichia coli DNA polymerase I deficient assay on 1-Naphthalene acetic acid (Pharmakon Laboratories, 7/25/78).

Test Material: 1-Naphthaleneacetic acid, Lot No. GN-2095

The purpose of this study is to determine if 1-naphthalene acetic acid (NAA) is more toxic to the E. coli DNA repair deficient strain p3478 than it is to the DNA repair competent strain W3110.

Method: E. coli strain W3110 and p3478 were grown to exponential phase in HAT broth. For the direct acting assay (without metabolic activation) the test compound was added at concentrations of 4, 2 and 1 mg/ml to sterile of man discs a a volume of 10 ul. To increase solubility the 4 mg/ml solution is in 10% DMSO. All other dilutions are made in distilled water. For metabolic activation assay 50 ul of S-9 mix were added to the test wells cut in the center of the petri plates.

Results: NAA did not produce a zone of inhibition in either strain. The lack of a zone of inhibition can be the result of the inability of the test chemical to diffuse through the media or its inability to produce genetic effects. These two alteratives may be resolved through the suspension assay. However, NAA has been shown to be water soluble and have little toxicity suggesting NAA does not produce genetic effects in E. coli.

Conclusion: NAA is non-mutagenic in this assay.

Classification: Core-Minimum Data

5. Ames Salmonella/microsome plate test (with and without metabolic activation on 1-Naphthalene acetic acid (Pharmakon Laboratories, 5/11/78).

The purpose of this study was to evaluate 1-Naphthalene acetic acid (NAA) in the Ames Salmonella typhimurium assay with and without metabolic preparation activation.

Method: NAA was dissolved in DMSO. Dose levels for preliminary toxicity screen were 5000, 1000, 200, 40 and 8 ug/plate. Strain TA100 showed partial inhibition at 5000 and 1000 ug/plate with very slight inhibition at 200 ug/plate. Strain TA1535 showed partial inhibition at 200 ug/plate and very slight inhibition at 1000 ug/plate.

Dose levels for NAA in the plate assay, with and without metabolic activation, were 5000, 1000, 200, 40, 8, 2, and 0.5 ug/plate. There were 0.07 ml of S-9 supernatant/1.0 ml S-9 mix used in the rat liver microsomal activation system.

Results: NAA was negative in strains TA1535, TA1537, TA1538, TA98 and TA100 of S. typhimurium in the Ames test.

Conclusion: NAA is non-mutagenic in this assay.

Classification: Core-Minimum Data

6. Micronucleus Test on 1-Naphthaleneacetic acid (Pharmakon Laboratories, 1/30/79).

The purpose of this study was to calculate the ability of 1-Naphthalene acetic acid (NAA) to produce chromosome breaking effects in mice pretreated with the chemical.

Methods: In a preliminary dose range study, NAA was administered once daily for two days to groups of 10 mice at concentrations of 250 and 125 mg/kg. No overt symtomatology or mortality was observed at 125 mg/kg and was chosen as the high dose. NAA was administered i.p. once daily for two days to four male and four female mice at the high dose of 125 mg/kg, and to a similar group of animals at a low dose of 60 mg/kg.

Concurrently, triethylenemelamine at 0.5 mg/kg as a positive control, and water at 20 ml/kg as a negative control, were administered to two groups of eight mice. All the animals were sacrificed by the inhalation of CO_2, six hours after the second dose.

Results: NAA was negative in the micronucleus test based upon the chemical's inability to produce a statistical;ly significant increase in the number of micronuclei per 1000 polychromatic RBC in the treated versus the control animals.

Conclusion: NAA is non-mutagenic in this assay.

Classification: Core-Minimum Data

7. Dominant Lethal Study with 1-Naphthaleneacetic acid ((Pharmakon Laboratories, 2/28/79).

Test Material: 1-Naphthaleneacetic acid (NAA)

Three groups of ten male Sprague Dawley COBS CD (SD) rats were administered NAA orally at doses of 500, 250 and 125 mg/kg.

Concurrently, triethylenemelamine (TEM), the positive control compound, was administered orally to a group of ten male rats at 0.8 mg/kg. Another group of ten male rats received 0.25% methylcellulose at 20 ml/kg and served as the negative control. Each substance was administered once daily for five consecutive days. Twenty-four hours after the fifth dose each male was co-housed with two virgin females for seven days. The matings were repeated weekly with two virgin females for a total of eight weeks. The females were sacrificed 14 days from mid-week lutea and live and dead implants were counted and recorded.

Results: NAA did not produce dominant lethal effects in the male rats at doses administered as measured by pre-implantation and post-implantation lossess. Post-implantation fetal deaths were significantly increased after the first four matings in those groups mated to male rats receiving TEM, the positive control.

Conclusion: NAA is not a dominant lethal chemical in this study.

Classification: Core-Minimum Data

8. Teratology Study with NAA (technical) by gavage in the Albino Rat (Huntingdon Research Center; HRC #R-42k6-46k-350), 1/14/77).

Test Material: 1-Naphthalene acetic acid (NAA)

Ninety-six (96) healthy timed pregnant CD rats were ordered from CRBL to be used as test animals. The experimental design is shown below:

Groups	Treatment	No. Female Rats
I	0 (control)	24
II	10 mg/kg NAA	24
III	50 mg/kg NAA	24
IV	250 mg/kg NAA	24

The day of mating as judged by the appearance of sperm in the vaginal smear, is considered day 0 of gestation. Dosing was started on day 6 and continued daily up to and including day 15 series of 0.05% sodium carboxymethylcellulose so that all animals were dosed by intragastric intubation at a standard volume of 1 ml per 100 mg body weight. Control animals were dosed in an identical manner with carboxymethylcellulose.

The dams were observed daily for signs of toxicity and weighed on day 1, 3, 6-15, 17 and 20 of gestation.

On day 20 of gestation the dams were euthanized with ether and the ovaries and uterine contents were immediately examined. One-third of the pups were preserved in Bouin's solution for subsequent free hand sectioning to ascertain possible visceral anomalies via Wilson's technique.

The remaining two-thirds were preserved in alcohol for subsequent dissection and examination under magnification followed by clearing and staining of the skeleton with alizarin and to detect possible skeletal anomalies.

Results: There were no deaths during the study nor any signs of toxicity due to treatment with NAA at any dose level. The group mean body weight in dams demonstrated a trend towards decreased body weight gains with the administration of NAA.

This decreased body weight gain was statistically significant in the group receiving 250 mg/kg of DAA which was evident from the onset of compound administration. While dams receiving either 10 or 50 mg/kg of DAA did not show a significant decrease in body weight gain during compound administration, the body weight gain from day 17 to 20 in these two groups lagged behind the body weight gain in the control group.

Litter size and fetal loss were not effected by treatment.

The incidences of major malformations and minor anomalies were compared in all groups.

Conclusion: NAA is not teratogenic in pregnant rats when given during days 6 to 15 of gestation at dosages of 250 mg/kg/day. At 250 mg/kg/day maternal decrease in body weight gained occurred with no increase in resorptions. The NOEL for maternal toxicity is 50 mg/kg/day.

Classification: Core-Minimum Data

9. Primary Eye Irritation of tech ethyl ester of NAA (AMR Biological Research, Contract No. 120-2148-113, 12/10/73).

Test Material: ethyl-NAA (tech)

0.1 gm of test material was instilled into one eye of each nine NZW rabbits with the untreated eye serving as a control. Three of the rabbits eyes were washed with approximately 20 ml of lukewarm water two seconds after instillation of test material and three rabbits eyes were washed similarly four seconds after instillation of the compound. The remaining three treated eyes were left unwashed. Scoring at 24, 48 and 72 hours after exposure.

Results: Washed and Unwashed eyes scored 0.0 on Draize scale.

Classification: Core-Minimum DATA

Toxicity Category IV: CAUTION

10. Acute Inhalation Study of Technical Ethyl Ester of NAA in Rats (AMR Biological Research, Contract No. 120-2148-113, 12/13/73).

One group of 6 male rats, 263-297 gms BW, were exposed to 206.5 mg/L of test material for one hour. Observation for 14 days.

Results: No deaths, LC_{50} > 206.5 mg/L

Toxic Signs: Irritation and lethargy

Body Weight: not reported

Necropsy: not remarkable

Classification: Core-Minimum DATA

Toxicity Category IV: CAUTION

11. Acute Dermal LD_{50} Test in Rabbits with Technical ester of NAA (AMR Biological Research, Contract No. 120-2143-113, 12/7/73).

Test Material: ethyl-NAA (tech)

One group of 4 male NZW rabbits received dermally a single dose of 5000 mg/kg of test material on the skin of the fur clipped trunk under an impervious cuff for 24 hours. Observation was for 14 days.
Results: No deaths, LD_{50} > 5000 mg/kg

Toxic Signs: none

Body Weight: not reported

Necropsy: not remarkable

Classification: Core-Minimum DATA

Toxicity Category III: CAUTION

12. Acute Oral Toxicity in Rats with Ethyl Ester of NAA (AMR Biological Research, Contract No. 120-2148-113, 12/6/73).

Test Material: ethyl-NAA (tech)

Three groups of 6 male rats, 199-285 grams BW, received doses of 2500, 5000 and 10,000 mg/kg of test material. Observation for 14 days.

Results: $LD_{50} = 3580 \pm 333$ mg/kg

Toxic Signs: Death

Body Weight: not reported

Necropsy: Stomach and intestines hemorrhagic, and filled with gas and fluid, lungs hemorrhagic, spleen dark in color, kidneys blanched, liver mottled, mesenteric blood vessels dilated, genital area wet, nose bloody.

Classification: Core-Minimum DATA

Toxicity Category III: CAUTION

13. Acute Dermal LD_{50} Test in Rabbits with AMCHEM-72-A112 Emulsifiable Concentrate 79068 (AMR Biological Research, Contract No. 120-2148-113, 12/7/73).

Test Material: AMCHEM-72-A112 E.C. 79068

One group of four male NZW rabbits received dermally a dose of 5000 mg/kg of test material on the skin of the fur clipped trunk under an impervious cuff for 24 hours. Observations for 14 days.

Results: No deaths, LD_{50} > 5000 mg/kg

Toxic Signs: none

Body Weight: not reported

Necropsy: Eschar formation at site of application.

Classification: Core-Minimum DATA

Toxicity Category III: CAUTION

14. Primary Eye Irritation of AMCHEM 72-A112 E.C. 79068

Test Material: AMCHEM-72-A112 E.C. 79068

0.1 ml was instilled into one eye of each of nine NZW rabbits with the untreated eyes serving as control. Three of the rabbit eyes were washed with 20 ml og lukewarm water two seconds after instillation; and three of the rabbit eyes were washed similarly four seconds after instillation. The remaining three treated eyes were left unwashed. Scoring at 24, 48, 72 hours and 7 days according to Draize.

Results: Corneal opacity, iritis and conjunctivitis in washed and unwashed eyes at day 7.

Classification: Core-Minimum DATA

Toxicity Category I: DANGER

15. Acute Inhalation Study of 72-A112 E.C. in rats (AMR Biological Research, Contract No. 120-2148-113, 12/10/73).

Test Material: AMCHEM-72-A112 E.C. 79068

One group of six male rats, 247-371 gm BW, were exposed by inhalation to 217.1 mg/L of test material for one hour. Observation for 14 days.

Results: No deaths, LC_{50} >217.1 mg/L

Toxic Signs: none

Body Weight: not reported

Necropsy: not remarkable

Classification: Core-Minimum DATA

Toxicity Category IV: CAUTION

16. Acute Oral Toxicity in Rats with Amchem 72-A112 - E.C. 79068 (AMR Biological Laboratories, Contract No. 120-1248-113, 12/6/73).

Test Material: AMCHEM-72-A112 - E.C. 79068

Three groups of 6 male rats received oral doses of 2500, 5000, and 10,000 mg/kg of test material. Observation for 14 days.

Results: LD_{50} = 5585 ± 760 mg/kg

Toxic Signs: death, hypoactivity

Body Weight: not reported

Necropsy: Bloody nose, stained genital area, stomach and intestine filled with gas and fluid; lungs hemorragic, kidneys blanched, spleen mottled.

Classification: Core-Minimum DATA

Toxicity Category IV: CAUTION

17. Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice; April 29, 1969; Innes, J.M.R. et al; Journal of the National Cancer Institute.

Compound No. 108 is 1-Naphthaleneacetic acid which was negative for oncogenicity in mice at ~~the~~ dose of 517 PPM (215 mg/kg)

Classification: Core-Minimum DATA

13. Ten-Day Range Finding Study with NAA by daily gavage to Rats (Huntingdon Research Center, HRC #R-4216-4, 8/16/76).

Test Material: NAA (tech)

NAA was administered by gavage to 6 rats (3M &3F) daily for 10 days at dose levels of 250, 1000 and 4000 mg/kg. Signs of ill health or toxicity were recorded daily. Body weights were recorded initially and at 2, 4, 6, 8 and 10 days after treatment began. Food consumption were recorded on days 2, 4, 6, 8 and 10 for each rat. All animals were necropsied which survived or succumbed during the study.

Results: Observations of the reactions of rats receiving the test material revealed the following symptoms: dyspnea, ataxia, lethargy, prostation, death after 2-3 days.

The deaths in groups receiving 1000 or 4000 mg/kg/day appear to be dose-related and are likely the result of compound toxicity.

The single death in the 250 mg/kg/day group after 9 days is probably due to inadvertant pulmonary gavage.

Examination of body weight data and food consumption data revealed a dose-related decrease in both body weight gain and food consumption when compared to the untreated control. The lack of homogenicity of time between the observations. Necropsy of animals which died during the study and all surviving animals at the end of the 10 day treatment period demonstrated an increase in various observations, such as discoloration of the lungs, liver and kidneys, as well as the presence of compound, blood and gas in the G.I. tract, when treated groups were compared to controls. No definitive dose-related patterns were discernible, however.

Conclusion: NO NOEL for the study.

Classification: Core-Minimum Data

19. Ninety-Day Toxicity Study in Rats with Technical NAA (CDC Research, Inc., 3/10/79).

Test Material: technical NAA

Two hundred Sprague-Dawley rats, 100 males and 100 females were used in the experiment.

The males had a mean body weight of 433-458 gms and the females were between 249-260 gms. The rats were allocated to four groups as shown below:

Group	Dose (mg/kg)	Initial Male	Initial Female	Lab Studies & Necropsies Day 30	Lab Studies & Necropsies Day 90
I Control	0	30	30	10 - 10	20
II Low	50	20	20	0 - 0	20
III Mid	150	20	20	0 - 0	20
IV High	300	30	30	10 - 10	20

Individual body weight and food consumption were recorded weekly, including the week prior to start of treatment. Actual intake of test compound was calculated weekly. The rats were observed daily for pharmacologic and toxicologic effects, and they were examined weekly for tissue masses and swellings.

Laboratory studies were performed pretest, at one month and at termination. Values for hemoglobin, hematocrit, RBC, total and differential WBC were obtained from tail blood samples from all animals pretest, from 10 males and 10 females each in control and high dose groups at one month, and from all surviving rats (except one) on days 82, 83 and 84. Likewise, mean corpuscular volume was determined at the same time. Values for glucose, creatinine, serum alkaline phosphatase, BUN, SGPT, total protein, albumin, globulin, and A/G ratio were recorded from five males and five females fasted rats in each of the control and high dose group at one month and from all surviving rats, which were fasted, at termination. The blood samples were drawn by cardioientesis on days 91, 92 and 93. A voided urine sample from the same number of rats was analyzed on days 30 and 82 for color appearance, sp. gr., protein, bilirubin, glucose, ketone, pH and occult blood.

A necropsy was executed on 10 male and 10 female fasted rats in Control and High dose groups on days 29 and 30, and on all surviving rats on Days 91, 92 and 93. Likewise a rat that died was necropsied. The following organs were weighed: heart, spleen, liver, adrenals, pituitary, testes, ovaries, kidneys and brain. All organs and tissues specified in the protocol were placed in 10% neutral buffered formalin, and they were processed by conventional methods for histopathologic examination and 30 tissues per animal were examined microscopically.

A section of liver from selected rats was placed in Carnoy's fluid for glycogen staining, if needed. A report detailing the results of the 30 day sacrifice was submitted to sponsor.

Results: A male control died on the 83rd day of test and necropsy revealed lesions charcteristic of leukemia. No abnormal behavior or pharmacologic or toxic effects were observed in the treated rats. No tissue masses were detected. Fluctuation in food consumption occurred in control and treated rats, and at several periods consumption by treated animals exceeded controls. However, the amount of food consumed by the high dose rats was imprecise because the animals ejected as unpalatable some of the diet mixture from the feeder.

Males administered the low and middle treatment levels gained at a slightly slower rate than controls and those given the highest level were moderately retarded in rate. Likewise, females in the low and middle treatment groups made slightly slower gains in body weight than controls, and high level group females gained only one-third the body weight acheived by controls.

All hematologic values were within the reference limits but there were slightly reduced values for hematocrit, hemoglobin, and/or RBC in the middle and high level males and females not considered compound-related.

Blood chemistries showed a trend toward higher values in alkaline phosphatase in the high dose group, probably associated with the rate of body growth.

There were no significant differences between control and treated rats in urinanalysis.

No grossly visible alterations were detected at necropsy in the control or treated male rats except for a control rats that showed an enlarged spleen and liver and red, depressed areas in the stomach.

Twenty-one of the females had an alteration. Clear fluid in the uterus (hydrometra) was noted in three controls, two low dose, seven mid-dose and five high-dose.

However, this condition in rats is usually not pathologic but represents a phase of the estries cycle. A mid-dose female and ovarian cysts and another, in the same group, exhibited inflammation of one eye. Focal omental fat necrosis was seen in one high-dose female and a nenocortical cyst in another of the high-dose females. All lesions observed are commonly encountered in the rat and none was judged compound induced.

The absolute and relative liver weight in high dose females appeared to be in the significant range. Other absolute and relative organs were comparable among groups.

Microscopic examination did not reveal a histologic basis for the heavier liver in high-dose females. The heavier liver in high dose females probably resulted from increased metabolic burden.

Conclusion: The NOEL for the study is 150 mg/kg/day in the rat for 90 days. The LEL is 500 mg/kg/day and the effects consisted of decreased body weight in both sexes and liver enlargement in female rats.

Classification: Core-Minimum Data

20. Six-Month Oral Toxicity Study of Naphthalene Acetic Acid in Beagle Dogs (Elars Bioresearch Laboratories, Project No. 1395, 5/3/79).

Test Material: NAA technical; Lot No. 16388

Thirty-two purebred beagle dogs, equally divided by sex and approximately 3 months of age, were randomized into four groups as shown below:

Group	Daily Dose (mg/kg)	No. of Dogs
I	control - sham dose	4 Male, 4 Female
II	50 mg/kg	4 Male, 4 Female
III	150 mg/kg	4 Male, 4 Female
IV	300 mg/kg	4 Male, 4 Female

Doses were prepared in gelation capsule for each individual dog based on body weight and treatment design. The dogs were dosed orally once a day for at least 180 consecurive days.

Observations were made daily from eight days predose to the day before the individually dog was necropsied. Feed consumption was measured twice weekly and the two measurements were averaged to give the average weekly feed consumption for that week. Feed consumption measurements were not considered very accurate due to spillage. These calculations were done for each dog on a weekly basis throughout the study. All dogs were weighed once a week. The dogs were weighed the morning they were scheduled for necropsy. This final weight is recorded as the terminal weight.

Physical examinations were conducted at predose, three months and six months. This included body temperature, heart rate, auscultation and examination of mucous membranes. Body temperature and heart rate were also measured bimonthly throughout the study and performed at the time of the weekly weighing.

Ophthalmologic examinations were done at predose, three months, and six months.

Mydriacil drops were used to dilate the pupils prior to examination.

Specimens for clinical pathology were collected at 13 days and 2 days predose and every month for the six month duration of the study.

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Hematology parameters included: total and differential WBC, RBC, platelet count, hematocrit, hemoglobin, MCV, reticulocytes.

Clinical Chemistry determinations included: glucose, BUN, creatinine, total protein, albumin, globulin, A/G ratio, total bilirubin, direct bilirubin, cholesterol, calcium, phosphorus, sodium, potassium, chloride, alkaline phosphatase, SGOT, SGPT, and LDH.

Urine collection for urinanalysis was done 13 days predose and then at 3 months and 6 months. Urinanalysis determinations included color, sp. gr., pH, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, blood, WBC count, RNC count and the presence of epithelium, bacteria, and triple phosphate crystals.

For the above tests, the dogs were fasted for at least 12 hours prior to collection.

At the termination of the study, the dogs were sacrificed with an intraverous injection of sodium pentobarbital anesthetic solution followed by exsanguination.

All dogs were fasted for least 12 hours before sacrifice. Gross observations were performed and samples of the organs listed below were taken.

Adrenals (2)
Rib (at costo chondral junction)
Diaphram
esophagus
heart
large intestine
Lungs (with bronchii)
mammary glands (female)
muscle
trachea
ovaries or testes (2)
pituitary
parotid salivary gland (2)
small intestine
spleen
thymus
tissue mass (if present)
Urinary bladder
Any gross lesions (if present)

Aorta
Brain (three levels) cecum
epididymes (2)
gall bladder
Kidneys (2)
Liver
Lymphnodes (cervical and mesenteric)
tonsils (2)
nerve
pancreas
prostate (male)
Skin
spinal cord (2 levels)
Stomach
thyroids & parathyroids (2)
tongue
Uterus

The eyes and testes were fixed in Bouin's and all other organs were fixed in 10% buffered formalin solution. The following organs were trimmed in a uniform manner and weighed: heart, liver, kidneys (2), adrenals (2), thyroids (2) and parathyroids (2), brains, testes (2) or ovaries (2) and pituitary.

Touch preparations and smears were made of rib bone marrow, and peripheral blood smears were taken. These slides were stained with Wright's stain and examined.

Tissues submitted for histopathological examination were embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

Results: Daily observations began at one week pretest and continued until the dog was sacrificed. Toxic signs were first seen in some of the Group IV dogs at 4 months.

Toxic signs were described as: anorexia lasting several days, tenderness in the mouth while dosing, icteric and pale mucous membranes, steady loss in weight, letharge, an uncoordinated gait, dark urine, and dark stools when present. Two of four male dogs and all of females showed some or all of these effects by the end of the study. In addition to the above toxic signs, dog NV78 had a great amount of edema in his hind legs. This swelling progressed over three days until his legs were swollen to approximately the coxo femoral joint. This dog was sacrificed on March 6, 1979.

An idiopathic alopecia and sloughing patches of the tongue were noted in several of the dogs throughout the study, but no significant trends were noted.

On daily observation of the dog's appetite, no trend was noted in any group until 4 months into the study.

At four months, the Group IV dogs began showing toxic effects, one of which was anorexia. The anorexia and resulting weight loss occurred sporadically significant throughout the last two months and affected three of the females (LC78, NX78 and UT78) and two of the males (NU78 and NV78).

Body weight data showed the mean weight and weight gain for the Group IV females to be significantly lower than control females at two, three, four, five and terminal months. These animals also showed significantly lower weight gains relative to control females.

Several of the dogfs showed an increase in the size of their popliteal and/or mandibular lymph nodes at the six month examination, but this was noted in all four groups without any significant trend.

Also noted at the six month examination was icteric mucous membranes in four of seven Group IV dogs (three females, NX78, OY78, UT78 and one male, NU78) and in one of eight Group III dogs (one male, NU78).

Ophthalmologic examinations showed lesions at six months which could not be concluded to be dose-related. Two female dogs of Group IV NX78 and UT78) showed retinal edema and uveitis, whereas the other 29 dogs did not. NX78 may also have had a detached retina and a vessel medial to the optic disc losse in the vitreous humor. However, these observations were difficult to confirm.

Urinanlysis showed that two dogs, TO78 (Group 1, male) and RM78 (Group II, female) consistently showed a high WBV count, which may indicate chronic cystitis. It is unlikely that these findings are treatment-related.

The Group IV male dog (NV78) which was necropsied on March 6, 1979, had a urine sample collected on March 5, 1979.

The results showed a large amount of bilirubin, urobilinogen and a small amount of RBC and Blood. These determinations are considered to be treatment related effects.

Hematological parameters were within the normal limits for canines in the four groups.

A blood sample was collected for NV78 on March 6, 1979, before exsanguination. His hematology values showed a slightly increased WBC count with a relative and absolute neutophilia and lymphopenia. This would be indicative of a stress hemogram and as such could be related to the moribund condition of the dog, which in turn may be compound-related.

The clinical chemistry analysis showed the SGPT values at four months and six months for the Group IV females were elevated above the normal limits.

The four month determination was slightly elevated, and the six month's was twice the normal value.

The clinical chemistry results for NV78 showed several parameters that were not within normal limits. Protein, cholesterol, and glucose were below normal and the total bilirubin, direct bilirubin, alkaline phosphatase, SGOT, SGPT were greatly elevated.

Dose-related increases in relative weights of kidney occurred in both males and females of Group IV. Dose related increase in liver, adrenals, brain and heart occurred in Group IV females. Group II males had a increase in relative kidney weights and Group III females had an increase in relative heart weights.

It should also be noted that the mean terminal body weights of the Group IV females was significantly less than the control (Group I) females at $p < 0.01$.

Histopathological examination of the tissues showed there were no significant histopathological alterations that were attributable to the test material or dose regimen in any of the tissues examined from control dogs in treatment GroupI.

In treatment Group II, there were 2/8 dogs that had very slight evidence of pericholangitis that was similar in nature to the lesions in the livers of dogs in Group III and IV.

In treatment Group III, there was evidence of a very slight to moderate degree of hepatic insults in 7/18 dogs. This insult was characterized by pericholangistis, toxic degeneration of hepatocytes and hepatocellular hypertrophy. There were 2/8 dogs that had hyperkeratosis of the skin at the thoroacolumbar junction

In treatment Group IV, there was evidence of slight to severe degree of hepatic insult in 8/8 dogs. This insult was characterized by congestive pericholangitis, toxic degeneration of hepatocytes, centrilobular necrosis, periportal fibrosis, hepatocellular hypertrophy, and development of a hyperplastic nodule in one dog (NX78). Furthermore, there was evidence of squamoid metaplasia in the tracheal epithelium of 2/8 dogs and a slight degree of myocarditis in one dog. There were 4/8 dogs in Group IV that had very slight to slight degree of hyperkeratosis of the skin at the thorocolumbar junction.

Microscopic examination of liver tissue sections from all dogs in all 4 treatment groups stained for the presence of glycogen revealed results similar to those reported above, i.e., there was decreased glycogen storage and degenerative change in treatment groups III and IV.

Conclusion: There is no NOEL for the 6-month dog study. The low dose group (50 mg/kg/day) showed slight evidence of pericholangitis in the liver of 2/8 dogs that was similar in nature to lesions in the livers of dogs in the mid-dose and high-dose groups.

Classification: Core-Minimum DATA

TOX/HED:th:RD Initial WWOODROW:11-15-79