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

APR 29 1987

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Naphthalene: 90-Day dermal toxicity study with rats (EPA ID No. 4413-1) 3/27/87

FROM: Krystyna K. Locke, Toxicologist
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THRU: Edwin Budd, Section Head
Review Section II
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TB Project No. 7-0447 Tox. Chem No. 587

Toxicology Branch/HED has completed an evaluation of the following study: "Ninety-Day (Sub-Chronic) Dermal Toxicity Study with Naphthalene in Albino Rats". No. 49-539 Revised, dated November 26, 1986. Review is attached.

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Reviewed by: Krystyna K. Locke
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Budd 4/29/87

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Dermal Toxicity

TOX. CHEM. NO.: 587

Accession NUMBER:

MRID NO.: 40021301

TEST MATERIAL: Naphthalene (tech. grade)

PROJECT NO.: 7-0447

SYNONYMS: None

RECORD NO.: 138987

STUDY NUMBER(S): 49-539 Revised

EPA ID NO.: 4413-1

SPONSOR: Texaco Chemical Company, Bellaire, TX

TESTING FACILITY: Bushy Run Research Center, Export, PA

TITLE OF REPORT: Ninety-Day (Sub-Chronic) Dermal Toxicity Study with Naphthalene in Albino Rats

AUTHOR(S): S.W. Frantz, J.P. Van Miller and W.C. Hengler

REPORT ISSUED: November 26, 1986

CONCLUSIONS:

Naphthalene, at all levels tested, had no effect on mortality, food consumption, body weight gains, hematology, clinical chemistry (most parameters), urinalysis, organ weights (excluding testes), and ophthalmology. Neoplastic lesions were not observed.

NOEL = 300 mg/kg/day
LEL = 1000 mg/kg/day; highest dose tested (excoriated skin and papules in both sexes; increased BUN and atrophy of seminiferous tubules in the males; and non-neoplastic lesions involving cervical lymph node, liver, thyroid, kidneys, urinary bladder, and skin - in the females).

Classification: Core- Guideline

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: Technical grade naphthalene.
Description: Clear flake solid of variable particle size.
Batch No.: 5601-56-1
Purity: 99.98%

2. Test animals:

Species: Rats
Strain : Sprague-Dawley
Age: Seven weeks at first dose.
Weight: 219.8 to 277.4 g (males) and 152.7 to 197.2g (females).
Source: Charles River Breeding Laboratories, Inc., Kingston, NY.

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned by a computer- generated randomization procedure to the following test groups.

Group	Number of Animals Male	Number of Animals Female	Dose Level of Naphthalene (mg/kg/day)
1 Cont.	20	20	0
2 Low	10	10	100
3 Mid	10	10	300
4 High	20	20	1000

All of the survivors were sacrificed after 13 weeks of dermal exposure. The additional ten animals per sex in the control and 1000 mg/kg groups were used for a recovery phase of four weeks (during which test material was not applied) and were sacrificed after test week 17.

The test material was applied as a neat solid under occlusion for 6 hours per day, 5 days per week. The application site was a clipped dorsal area of the trunk. Following each exposure, the treatment area was wiped clean, and the wrap and the test material discarded.

Dose were selected by the sponsor and were based on preliminary studies conducted elsewhere. Also, 1000 mg/kg/body weight is a maximum dose that can be feasibly held in contact with the skin.

The rats were housed singly before and during dosing.

2. Diet preparation - Commercial Ground Purina Certified Rodent Chow #5002 was used as purchased. Both the diet and the test material were stored at temperatures of 68° to 75°F and relative humidity of 30% to 70%. The test material was analyzed for stability at 30-day intervals.

Results - The test material was stable under the above storage conditions.

3. Animals received food and water ad libitum during the non-dosing periods. Food was withheld during the dosing periods.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

"Food consumption, body weight, and organ weight data were intercompared for the dose groups and control group by use of Levene's test for homogeneity of variances[1], by analysis of variance[2], and by individual t-tests[2]. The t-tests were used, if the analysis of variance was significant, to delineate which groups differed from the control group. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variances[3] followed, if necessary, by individual t-tests. The fiducial limit of 0.05 (2-tailed) was used as the critical level of significance for all tests."

REFERENCES

1. Brown, M. B. and A. B. Forsythe (1974), J. Amer. Statis. Assoc., 69, 364-367.

2. Sokal, R. R. and F. J. Rohlf (1969), Biometry, W. H. Freeman and Company, San Francisco.

3. Brown, M. B. and A. B. Forsythe (1974), Technometrics, 16, 129-132.

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5. Quality assurance was conducted by the testing laboratory on twelve occasions between 12/17/85 and 11/25/86. The results of each inspection were reported by the Quality Assurance Unit to both the study director and management.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected daily for signs of toxicity and mortality. Detailed clinical observations were performed once each week.

Results:

Toxicity - The only toxic sign observed was an increased incidence of excoriated skin and papules in the treatment area of the high-dose males and females. This condition persisted throughout most of the study.

Mortality (survival) - There were four treatment-unrelated deaths. The following animals were found dead:

Group	Sex	Days on test	Cause of death
Control*	Male	121	Unknown
High-dose	Male	57	Urinary tract infection
Control	Female	3	Stress from dosing
Low-dose	Female	1	Stress from dosing

*This animal was from the recovery group and died shortly before it was to be sacrificed at the termination of the recovery period.

2. Body weight - The rats were weighed weekly throughout the study and mean weekly body weights were calculated.

Results - Naphthalene, at the levels tested, had no effect on body weight gains when the treated animals were compared with the controls.

3. Food consumption - Consumption was determined weekly and mean weekly diet consumption was calculated. Food efficiency was not calculated.

Results - Naphthalene had no effect on food consumption in this study. Although the high-dose males consumed from 7.3% to 25.2% ($P = 0.05$ or 0.01) more food than did the controls during the treatment weeks 2, 3, 4, 9 or 13, these increases did not result in body weight changes and were considered spurious. Similar increases in food consumption, but no weight changes, were noted in this group during the recovery period (test weeks 14 through 17).

4. Ophthalmological examinations were performed, using an indirect ophthalmoscope, on all animals prior to dosing and prior to final sacrifice.

Results - The ophthalmological findings were minimal and were not considered to be treatment-related. The following findings were observed at the termination of the study: bilateral retinal atrophy or hypervascularization in two low-dose males; conjunctivitis in one mid-dose male; mild keratosis in one control female; and severe keratoconjunctivities in one low-dose female. Similar findings were observed in two males (different from those above) and five females (different from those above) during the predosing examination.

5. Blood was collected for hematology and clinical analyses as follows: before treatment and prior to sacrifice during week 13 (from 10 rats/sex/group); and at 4 weeks (from 5 rats/sex/group). All animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)

Results - Naphthalene, at all levels tested, had no effect on any of the above parameters examined.

b. Clinical Chemistry

	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*		Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine Phosphokinase*	X	Direct Bilinubin
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

Results - Naphthalene had no effect on the above parameters examined. Although changes were observed in some parameters, they were inconsistent and could not be supported histologically, and were therefore regarded as spurious and of unclear biological significance. The following changes were observed when the treated animals were compared with the controls:

At 4 Weeks:

- Dose-related increase in blood urea nitrogen (BUN) of the male rats. The increases in BUN in each of the three treatment groups were 6%, 12% and 35% greater than controls, respectively, but were statistically insignificant.

- Statistically significant increases in creatinine of the low-dose (17% increase)[a] and mid-dose (33% increase)[b] female groups.

- Statically significant[b], although dose-unrelated, decreases in serum aspartate aminotransferase (AST) concentrations of the female groups. These decreases were 18%, 19% and 17% in the low-dose, mid-dose and high-dose groups, respectively.

- Dose-related decreases in phosphorus serum concentrations of the females. These decreases were 7%, 16%[a] and 22%[b] in the three treated groups, respectively.

AT 13 Weeks:

The concentrations of serum AST and alanine aminotransferase (ALT) were decreased in the female rats when compared with those of the controls. AST values were decreased by 18%, 23% and 14% in the low-dose, mid-dose and high-dose groups, respectively. The corresponding decreases for the ALT values were 30%, 36% and 12%, respectively. All of the decreases were statistically insignificant.

A comment was made that the above decreases were due to two animals in the control group that had values outside the historical range for these determinations. When these two animals were removed from the evaluation, the AST and ALT values in the treated rats did not differ from those of the controls:

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$

6. Urinalysis - Urine was collected from fasted animals before dosing and at weeks 4 and 13 during dosing. The CHECKED (X) parameters were examined.

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

Results - Napthalene had no effect on the above parameters examined.

7. Sacrifice and Pathology
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

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	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. 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 n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland
X	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver*	X	Epididymides	X	Thyroids*
	Gall bladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin
X	Lung	X	Vagina	X	All gross lesion and masses

Histologic examinations were performed on all tissues from animals in the control and 1000 mg/kg/day dose groups at 13 weeks. The lungs, liver, kidneys and tissues with gross lesions were examined in animals from the 100 and 300 mg/kg/day dose groups.

Tissues were also examined histologically from all rats dying during the study, but organ weights were not obtained on these animals.

Results

a. Organ weight - Absolute organ weights, organ weights as percent of final body weight and as percent of brain weight were obtained.

Naphthalene had no effect on the absolute and relative weights of brain, liver, kidneys, heart, adrenals and ovaries in the low-dose and high-dose groups of both sexes. The following organ weights were statistically significantly different from those of the controls:

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- o Decreased absolute and relative weights of testes in the high-dose males at 13-week sacrifice. These decreases were 7% ($p < 0.05$) and 9% (relative to brain weight; $p<0.01$), respectively, and were regraded as being of negligible biological significance for the following reasons: (1) the weight differences were small and (2) the tissue was histologically normal.

- o Increased relative weights (calculated as percent of final body weight) of liver, kidneys and adrenals in the mid-dose male group at 13-week sacrifice. These increase were 10% ($p < 0.01$), 7% ($p < 0.05$) and 14% ($p < 0.05$), respectively, and were regarded as spurious due to (1) the absence of a dose-response relationship and (2) the absence of abnormal histopathology.

b. Gross pathology

No treatment-related gross lesions were observed in this study. At 13-week sacrifice, the predominant lesions noted at all levels were "color change" and "size change" of the cervical lymph node in males and females, and excoriated skin and alopecia in the females.

The only treated (low-dose) female which was found dead on test day one had eye opacity and "size change" of the cervical lymph node.

The only treated (high-dose) male which was found dead on test day 57 had hydronephrosis, renal calculi, distended urinary bladder, granular material in urinary bladder, and "size change" "and/or "color change" of kidneys, ureter, and cervical and mediastinal lymph nodes.

The only control female, found dead on day 3, had "color change" of the liver and "size change" of the cervical lymph node. The only control male, found dead on day 121, had emaciated body, encrusted nose/turbinates, and "size change" of the spleen.

c. Microscopic pathology

1) Non-neoplastic

The predominant non-neoplastic lesions observed in the male rats at 13-week sacrifice were bile duct and lymphoid (cervical node) hyperplasia, mineralization of the pulmonary vessels, acanthosis (treated skin) and thyroglossal duct cyst(s) in the thyroid. However, the incidence of these lesions (number of tissues affected/

number of tissues examined) was either the same or higher in the control than in the naphthalene-treated rats. Only seminiferous tubule atrophy of the testes was higher in the high-dose males (2/10) than in the controls (0/10).

The incidence of predominant non-neoplastic lesions observed in the female rats at 13-week sacrifice was generally higher in the high-dose group than in the controls, as follows:

Lesion	Incidence	
	Controls	High-dose
Lymphoid hyperplasia (cervical node)	4/10	7/10
Thyroglossal duct cyst(s) (thyroid)	5/10	8/10
Hemosiderosis (liver)	1/10	4/10
Pyelonephritis (kidneys)	0/10	2/10
Transitional cell hyperplasia (urinary bladder)	0/10	1/9
Acanthosis (treated skin)	1/10	3/10
Acanthosis (untreated skin)	1/10	4/10
Hyperkeratosis (untreated skin)	0/10	3/10

Only the degeneration of sternal cartilage was slightly higher in the control females (5/10) than in the high-dose females (4/10).

The following non-neoplastic lesions were observed in the four rats which died during the course of the study:

- o Lymphoid hyperplasia (cervical node) in one male and one female from the control group.
- o Lymphoid necrosis and hemorrhage in the thymic region and cystitis of the urinary bladder - in the high-dose male.
- o Lenticular cataract, mononuclear cell infiltrates, and bile duct and cervical node hyperplasia - in the low-dose female.

2) Neoplastic

Neoplastic lesions were not observed in this study.

D. DISCUSSION:

Comments - This is a well-planned and well-reported study, although some gross pathology data, such as size change of the cervical lymph node, should have been more specific. However, this is not critical because one learns from the microscopic pathology section what was meant by "size change".

NOEL = 300 mg/kg/day
LEL = 1000 mg/kg/day (highest dose tested)

Classification: Core-Guideline

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*Recommended by Subdivision F (Oct. 1982) guidelines for chronic studies.