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WASHINGTON, D.C. 20460

CASWELL FILE

011753

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

**SUBJECT:** Naphthalene: 82-4 Subchronic (90-Day) Inhalation  
Study in the Rat

Submission No. S447887	DP Barcode No. D194970
EPA ID Number: 055801	P.C. Code No. 0.55801
Rereg. Case No. 0022	Case No. 818942
CAS Registry No. 91-20-3	EPA Regis. No. 43841-1
Tox. Chem. No. 587	

**FROM:** Krystyna K. Locke, Toxicologist  
Section I, Toxicology Branch I  
Health Effects Division (7509C)

*Krystyna K. Locke 10/12/95*

**TO:** Larry Schnaubelt / Beverly Lavis, PM Team No. 72  
Reregistration Branch  
Special Review and Reregistration Division (7508W)

**THRU:** Roger Gardner, Section Head  
Section I, Toxicology Branch I  
Health Effects Division (7509C)

*Roger Gardner 12/15/95*  
*12/8/95*

Section I, Toxicology Branch I/HED has completed an evaluation of the following study:

Naphthalene: 13-Week Inhalation Study in Rats; D.W. Coombs, P.C. Kieran, C.J. Hardy, D. Crook, D.J. Lewis and C. Gopinath; Hutingdon Research Centre Ltd., England; Report No. LDA 2/930704; Study Completion Date: April 28, 1993.  
MRID No. 42835901

In this study, male and female Sprague-Dawley rats were exposed to naphthalene vapor for 13 weeks (snout only, 6 hours/day, 5 days/week). The nominal concentrations of naphthalene in the exposure chambers were 0 (air control), 2, 10 or 60 ppm (0, 0.01, 0.052 or 0.315 mg/L, respectively). The analytical concentrations of naphthalene in the exposure chambers were 0, 0.011, 0.051 or 0.306 mg/L, respectively.

Compared with the controls, treatment-related effects were observed in all groups. In the low-dose group (0.01 mg/L), male and female rats had degenerative changes and proliferative lesions, classified as minimal, in the nasal passages.



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The above lesions, classified as moderate, hypertrophy of the respiratory epithelium, and decreased body weight gain and food consumption (males only) were observed in the mid-dose group (0.052 mg/L).

The following effects were observed in the high-dose (0.315 mg/L) male and female rats: (1) Histological changes (like those in the mid-dose group), classified as marked; (2) Degenerate (minimal) fibers in the spinal cord and sciatic nerve (one male); and (3) Decreased body weight gain and food consumption.

Based on the above findings, the systemic NOEL for both sexes is < 2 ppm (0.01 mg/L), LDT. This study is classified as Acceptable and satisfies the guideline requirement 82-4 for a subchronic inhalation toxicity study in the rat.

Krystyna K. Locke 10/12/95

**Primary Review by:** Krystyna K. Locke, Toxicologist  
Section I, Toxicology Branch I, Health Effects Division (7509C)

**Secondary Review by:** Roger Gardner, Section Head *Roger Gardner 12/8/95*  
Section I, Toxicology Branch I, Health Effects Division (7509C)

**DATA EVALUATION RECORD**

**STUDY TYPE:** 82-4 Subchronic Inhalation Toxicity (90-Day) in the Rat

**EPA IDENTIFICATION NUMBERS:**

MRID No. 42835901  
Submission No. S447887  
P.C. Code No. 055801  
Case No. 818942  
EPA Regis. No. 43841-1

DP Barcode No. D194970  
EPA ID No. 055801  
Rereg. Case No. 0022  
CAS Registry No. 91-20-3  
Tox. Chem. No. 587

**TEST MATERIAL:** Naphthalene; grey-white crystalline solid; stored at room temperature; lot no. LI-1 (LX No.: LX158-01)  
Purity: 99.9%

**REPORT NUMBER:** LDA 2/930704

**SPONSOR:** RECOCHEM, Montreal, Quebec, Canada

**TESTING FACILITY:** Huntingdon Research Centre Ltd., Cambridge-shire, England

**TITLE OF REPORT:** Naphthalene: 13-Week Inhalation Study in Rats

**AUTHORS:** Derek W. Coombs, Pearse C. Kieran, Colin J. Hardy, David Crook, David J. Lewis and Chirukandath Gopinath

**STUDY COMPLETION DATE:** April 28, 1993

## EXECUTIVE SUMMARY

In this subchronic inhalation toxicity study, Sprague-Dawley rats, 10/sex/group, were exposed to an atmosphere containing vapor of naphthalene (purity: 99.9%) for 13 consecutive weeks (snout-only, 6 hours/day, Monday through Friday). The target concentrations of naphthalene in the exposure chambers were 0, 2, 10 or 60 ppm (0, 0.010, 0.052 or 0.315 mg/L, respectively) and were based on the results observed in a 28-day range-finding study (No. LDA 1/921-559; not submitted). The analytical concentrations of naphthalene in the exposure chambers were 0, 0.011, 0.051 or 0.306 mg/L, respectively. Treatment-related effects were observed in all groups.

In the low-dose group (0.01 mg/L), 30-90% of the male and female rats had degenerative changes and proliferative lesions, classified as minimal, in the olfactory epithelium, and the loss of Bowman's glands. These effects in the nasal passages were not observed in the control group.

The above lesions, classified as moderate, plus hypertrophy of the respiratory epithelium, were also observed in 30-100% of the mid-dose (0.052 mg/L) rats. Compared with the controls, decreased body weight gain for the males (30%,  $P < 0.01$ ) and the females (18%), and decreased food consumption for the males (13%) throughout the study were also observed in this group.

The following effects were observed in the high-dose (0.315 mg/L) rats when compared with the controls: (1) Histological changes (like those in the mid-dose group), classified as marked, in 40-100% of the males and females; (2) Degenerate (minimal) fibers in the spinal cord of 10% males and 10% females, and in the sciatic nerve of 10% males; (3) Decreased body weight gain throughout the study (males, 42% and females, 34%;  $P < 0.01$ ); and (4) Decreased food consumption throughout the study (males, 14%,  $P < 0.05$  and females, 9%).

Based on the above findings, the systemic NOEL for both sexes is  $< 2$  ppm (0.01 mg/L), LDT. This study is classified as Acceptable and satisfies the requirement, § 82-4, for a sub-chronic inhalation toxicity study in the rat.

## EXPERIMENTAL PROCEDURES

This study was conducted from October 12, 1992 (first exposure) through January 11, 1993 (last exposure). The terminal sacrifice took place on January 11-12, 1993. This study was designed to comply with the U.S. EPA (FIFRA) Pesticide Assessment Guidelines, revised in November, 1984 (Guideline 82-4) and with the OECD Guidelines for Testing Chemicals No. 413.

Sprague-Dawley rats, 10 males and 10 females per group, were exposed to an atmosphere containing vapor of naphthalene for 13 consecutive weeks (snout-only, 6 hours/day, Monday through Friday each week). The target concentrations of naphthalene in the exposure chambers were 0 (Group 1), 2 (Group 2), 10 (Group 3) or 60 (Group 4) ppm [0, 0.010, 0.052 or 0.315 mg/L, respectively]. The analytical concentrations of naphthalene in the exposure chambers were  $0.011 \pm 0.002$ ,  $0.051 \pm 0.011$  or  $0.306 \pm 0.044$  mg/L for Groups 2, 3 and 4, respectively. Samples of test atmosphere were withdrawn for analyses on at least 3 occasions during each exposure. The concentrations of naphthalene used in this study were selected by the sponsor and were based on the results observed in a 28-day range-finding study (HRC Report No. LDA 1/921559; not submitted).

A separate vapor generation system was used for each group. At lower concentration of naphthalene (Group 2), the test atmosphere was generated under ambient temperature using the carrier airflow to maintain the concentration. At higher concentrations (Groups 3 and 4), the flasks containing naphthalene and the carrier air were heated in a water bath to aid vaporization. The control rats were exposed to air only. The air was supplied to the chamber at 25 liters/minute. The mean chamber oxygen concentration was 21% throughout the study. Each chamber was a 28 cm cylinder with a volume of approximately 50 liters. The rats were:

- (1) Obtained from Charles River Ltd., Portage, Michigan, U.S.A. on September 24, 1992.
- (2) About 6 weeks old at the time of purchase and weighed 200-201 g (males) and 141-142 g (females) at the allocation to groups on September 29, 1992.
- (3) Housed in groups of 5, of the same sex, in suspended stainless steel cages, at temperatures of 18-26°C, relative humidity of 30-67% and 12 hours light/12 hours dark cycles.
- (4) Acclimated for about 2 weeks prior to the start of treatment.

- (5) Allocated to groups, using a computer program, on the basis of body weights in such a manner that the group mean body weights were approximately equalized.
- (6) Identified by numbers tattooed on the ear pinnae.
- (7) Allowed free access to food (SDS Rat and Mouse No. 1 modified diet, Special Diet Services, Witham, Essex, England) and tap water (polypropylene bottles).

The following parameters were examined for all rats on the study unless indicated otherwise:

- (1) Clinical Observations: Were recorded twice daily and during exposure.
- (2) Body Weights: Were determined for each rat at the time of allocation to groups and at weekly intervals thereafter, until the termination of the study.
- (3) Food Consumption: Was recorded weekly by cage, starting one week before exposure and continuing up to and including the week of necropsy.
- (4) Hematology: The following determinations were performed during week 13 of the study: Packed cell volume, hemoglobin, red cell count, mean corpuscular hemoglobin concentration (calculated), mean corpuscular volume (calculated), total white cell count, differential cell count (neutrophils, lymphocytes, eosinophils, basophils, monocytes and platelets), reticulocyte count and thrombotest. Blood was removed from the orbital sinus under light ether anesthesia. The rats were deprived of food overnight.
- (5) Clinical Chemistry: The following determinations were performed during week 13 of the study: Creatine phosphokinase (creatine kinase), glucose hexokinase, glutamic-pyruvic transaminase (alanine aminotransferase), glutamic-oxaloacetic transaminase (aspartate aminotransferase), gamma-gutamyltransferase, alkaline phosphatase, total protein, albumin, globulin (calculated), urea nitrogen, total bilirubin, creatinine, sodium, potassium, calcium, inorganic phosphorus, chloride and cholesterol. Blood was removed from the orbital sinus under light ether anesthesia, placed into tubes containing anticoagulant and centrifuged at 3200 g for 3 minutes to obtain plasma for the above determinations. The rats were deprived of food overnight.
- (6) Ophthalmoscopy: Using a Keeler indirect ophthalmoscope, the eyes of all rats were examined before assignment to

groups and during test week 13.

- (7) **Necropsy:** At the end of the 13-week exposure, the rats were anesthetized by intraperitoneal injection of sodium pentobarbitone and killed by exsanguination from the bronchial arteries. A detailed macroscopic examination of each rat was performed.
- (8) **Organ Weights:** The following organs were weighed after exsanguination: Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes (with epididymides) and thymus.
- (9) **Histological Examination:** The tissues examined and the processing and fixation techniques used are listed in Attachment I of this review. All listed tissues were examined for Group 1 (Controls) and Group 4 (High-dose). The following tissues were examined for Group 2 (Low-dose) and Group 3 (Mid-dose): Nasal turbinates, lungs, liver and kidneys.
- (10) **Statistical Analyses:** Many procedures were used in analyzing the above data and these are detailed in Attachment II of this review.

## **RESULTS**

### **Clinical Observations**

Toxic signs were not observed in any group during exposure. At other times, there was an increased incidence of brown staining on body fur in groups exposed to naphthalene. The numbers of the male rats affected in Groups 1, 2, 3 and 4 were 0, 0, 1 and 7, respectively. The corresponding numbers for the female groups were 5, 4, 8 and 9, respectively. The staining was observed most frequently during weeks 11-14 in Group 1 females; during week 9 in Group 2 females; during weeks 9-14 in Group 3 females and Group 4 males; and during weeks 6-14 in Group 4 females.

### **Mortality**

Three rats died during the study, 2 males and 1 female, but none of these deaths was treatment-related. One control (Group 1) male died during week 14 because of an anesthetic accident during bleeding. One low-dose (Group 2) male was killed during week 4 due to injury to the right hind leg. The death of the low-dose female during week 13 was attributed to the effects of the tube restraint during exposure to naphthalene.



### Body Weights

Compared with the controls, decreased body weight gains were observed for the high-dose males and females, and for the mid-dose males throughout the study. These data are summarized below.

Body Weight Gains (g) During the Study						
Test Group	Week:	0-3	0-6	0-9	0-13	% of AC
Air control:						
Males		56	110	141	167	-
Females		26	46	52	62	-
Low-dose:						
Males		40	94	127	158	94.6
Females		15	31	39	53	85.5
Mid-dose:						
Males		32	63	94	116**	69.5
Females		20	34	41	51	82.2
High-dose:						
Males		27	57	79	96**	57.5
Females		13	27	32	41**	66.1

This table is based on TABLE 4, page 51, of the submitted report (MRID 42835901). \*\* P<0.01 compared with control values using Williams' test. % of AC = % of Air control for weeks 0-13

### Food Consumption

Compared with the controls, decreased food consumption was observed for the mid-dose and high-dose males throughout the study. Food consumption was also reduced for the high-dose females, but the difference from control was not statistically significant. These data are summarized below.

Food Consumption - Group Mean Cumulative Values (g)					
Test Group	Week:	1-3	1-6	1-9	1-13
Air control:					
Males		779	1356	1933	2670
Females		536	938	1326	1827
Low-dose:					
Males		725	1282	1849	2600
Females		510	899	1269	1752
Mid-dose:					
Males		675*	1153*	1653*	2317
Females		536	918	1283	1762
High-dose:					
Males		690*	1174*	1653*	2301*
Females		495	857	1207	1666

This table is based on TABLE 5, page 52, of the submitted report (MRID 42835901). \* P<0.05 compared with control values using Williams' test.

### Hematology

Total white cell (WBC) and lymphocyte (L) counts were decreased in all treated male groups and increased in all treated female groups. These were the only differences from control values achieving statistical significance. However, because these differences were not dose-related and not consistent between sexes, the testing facility (and Section I, Toxicology Branch I/HED) considered them to be of no toxicological significance. The WBC and lymphocyte data are summarized below.

Group	Control	Low-dose	Mid-dose	High-dose
WBC x 10 <sup>3</sup> /mm <sup>3</sup> :				
Males	14.8	11.9*	11.9*	11.3**
Females	5.7	8.4*	8.2*	7.3*
L x 10 <sup>3</sup> /mm <sup>3</sup> :				
Males	12.86	9.70**	9.72**	8.95**
Females	4.59	7.17**	7.07**	6.32**

This table is based on TABLE 6, page 53, of the submitted report (MRID 42835901). \* P<0.05 and \*\* P<0.01 compared with control values using Williams' test.

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Clinical Chemistry

Statistically significant differences from control values were observed mostly in the high-dose group. The parameters affected were glucose, total protein, albumin, globulin, alkaline phosphatase, sodium, potassium and chloride. However, because these differences were not dose-related and not consistent between sexes, the testing facility (and Section I/Toxicology Branch I/HED) considered them to be of no toxicological significance. The observed findings are summarized below.

Group	Low-dose	Mid-dose	High-dose
Finding	Rats Affected		
Glucose (↑)	.....	.....	..M.....
Total protein (↓)	.....	.....F..	..M...F..
Albumin (↓)	.....F..	.....F..	.....F..
Globulin (↓)	.....	.....	..M.....
Alkaline phosphatase (↑)	.....	.....	.....F..
Sodium (↓)	.....	..M.....	..M...F..
Potassium (↑)	.....	.....	.....F..
Chloride (↓)	.....	.....	.....F..

This table is based on TABLE 7, pages 54-55, of the submitted report (MRID 42835901). The actual values and statistical significance are in Attachment III. M = Males and F = Females (↑) = Increased and (↓) = Decreased

Ophthalmoscopy

Treatment-related findings were not observed. Hyaloid remnants in the vitreous body were observed before exposure to naphthalene in 2 control, 0 low-dose, 6 mid-dose and 2 high-dose male rats and in 5 control, 1 low-dose, 3 mid-dose and 1 high-dose female rats. After treatment, 1 control, 1 low-dose, 1 mid-dose and 2 high-dose males, and 1 control female had hyaloid remnants.

Necropsy

Relative to the control values, an increased incidence of fur staining was observed in the males and females, and of stomach antrum mucosa in the females, exposed to naphthalene. Because the incidence of fur staining was dose-unrelated in the females, and of stomach antrum mucosa in the males, it is difficult to attribute these findings unequivocally to treatment. The incidences of fur staining and stomach antrum mucosa are summarized below.

Group	Control	Low-dose	Mid-dose	High-dose
<b>Finding</b>	<b>Number of Rats with Finding</b>			
<b>Stained fur:</b>				
Males	0	0	4	7
Females	1	4	10	8
<b>Stomach antrum mucosa; white nodule(s):</b>				
Males	3	1	2	2
Females	2	2	3	6

This table is based on TABLE 8, pages 56-58, of the submitted report (MRID 42835901). All rats in the study (10/sex/group) were examined.

#### Organ Weights

Naphthalene, at all concentrations tested, had no effect on organ weights examined in this study.

#### Histological Examination

Treatment-related changes were observed only in the nasal passages, in all naphthalene-treated groups, and involved olfactory and respiratory epithelia, and the subepithelial Bowman's glands. These changes are summarized below.

Group	Control	Low-dose	Mid-dose	High-dose
	M/F	M/F	M/F	M/F
<b>Findings in Nasal Passages</b>	<b>Number of Rats with Findings</b>			
<b><u>Olfactory Epithelium - Degenerative Changes:</u></b>				
Atrophy	0/0	9/9	9/10	10/10
Erosion	0/0	1/0	4/5	6/5
Occasional degenerate cells	0/0	4/3	4/3	8/8
<b><u>Olfactory Epithelium - Proliferative Lesions:</u></b>				
Hyperplasia of basal cells	0/0	3/6	8/7	6/6
Rosette formation	0/0	3/3	7/7	4/6

Treatment-related changes in the nasal passages - continued

Group	Control	Low-dose	Mid-dose	High-dose
	M/F	M/F	M/F	M/F
Findings in Nasal Passages	Number of Rats with Findings			
<b><u>Respiratory Epithelium:</u></b>				
Hypertrophy	0/0	0/0	4/6	10/6
<b><u>Bowman's Glands:</u></b>				
Loss of glands	0/0	5/6	9/9	10/10

This table is based on TABLE 10, pages 61-62 of the submitted report (MRID 42835901). All rats in the study (10/sex/group) were examined. M/F = Males/Females

The severity of the above lesions increased with dose. Lesions observed in the low-dose group were generally classified as minimal; those in the mid-dose group, as moderate; and those in the high-dose group, as moderate and marked. No abnormalities were detected in the tongue, pharynx, trachea, tracheal bifurcation and lungs (in most rats). No abnormalities were also detected in the brain and, with three exceptions, in the spinal cord and sciatic nerve. One high-dose male and female had degenerate fibres (minimal) in the spinal cord, and another high-dose male had degenerate fibres (minimal) in the sciatic nerve.

**COMMENTS**

This study was conducted in compliance with the following Good Laboratory Practice (GLP) Standards:

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, November 29, 1983 and subsequent amendment, Federal Register August 17, 1989.

GLP, The United Kingdom Compliance Programme, Department of Health and Social Security 1986 and subsequent revision, Department of Health, 1989.

Japanese Ministry of International Trade and Industry, March 31, 1984.

Organization for Economic Co-operation and Development (OECD), ISBN 92-64-12367-9, Paris, 1982.

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This study was also designed to comply with the following guidelines: (1) U.S. EPA (FIFRA) Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation, Human and Domestic Animals (Revised) - November, 1984 (Guideline 82-4); and (2) OECD Guidelines for Testing of Chemicals No. 413. However, urinalysis, a supplemental criterion for a subchronic inhalation toxicity study (according the EPA ACCEPTANCE CRITERIA, December 24, 1989) was not performed.

With the exception of the purity of the test material (reported only as "assumed pure")\*, this study is well designed and well reported. All experimental/analytical procedures used were referenced and/or described. The following statements were also included in the report:

1. Statement of Data Confidentiality Claims
2. Quality Assurance Statement. This study was inspected 6 times during October 7, 1992 and January 13, 1993.

\* The purity of naphthalene (99.9%) was supplied by Landis International, Inc., Valdosta, GA. (Regulatory Agent for RECOCHEM, the registrant) via telefax on October 4, 1995 (Attachment IV in this review).

Attachment I

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Page 15 is not included in this copy.

Pages \_\_\_\_\_ through \_\_\_\_\_ are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
  - Sales or other commercial/financial information.
  - A draft product label.
  - The product confidential statement of formula.
  - Information about a pending registration action.
  - FIFRA registration data.
  - The document is a duplicate of page(s) \_\_\_\_\_.
  - The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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Attachment II

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Page \_\_\_\_\_ is not included in this copy.

Pages 17 through 18 are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
  - Sales or other commercial/financial information.
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REFERENCES

1. Bartlett, M.S., (1937), Proc. Roy. Soc. Series A, 160: 268 - 282.
2. Kruskal, W.H. and Wallis, W.A., (1952/3), J. Amer Statist. Ass., 47: 583 - 621 and 48: 907 - 912.
3. Williams', D.A., (1971/2), Biometrics, 27: 103 - 117 and 28: 519 - 531.
4. Shirley, E., (1977), Biometrics, 33: 386 - 389.

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Attachment III

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Page \_\_\_\_\_ is not included in this copy.

Pages 20 through 21 are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
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Attachment IV

011753



LANDIS INTERNATIONAL, INC.  
R & D Management

October 4, 1995

VIA TELEFAX [703-308-8773]

Ms. Beverly Lavis, Review Manager  
Reregistration Branch  
Special Review and Reregistration Division (H7508W)  
U.S. ENVIRONMENTAL PROTECTION AGENCY  
Office of Pesticide Programs  
Document Processing Desk  
2800 Crystal Drive  
Crystal Station 1, Third Floor  
Arlington, VA 22202

SUBJECT: NAPHTHALENE -- 13 WEEK RAT INHALATION STUDY  
MRID 42835901

REFERENCE: EPA REGISTRATION NO. 43841-1; PHONE CONVERSATIONS  
BETWEEN BEVERLY LAVIS AND RONALD LANDIS ON  
SEPTEMBER 25, 1995, AND OCTOBER 4, 1995

Dear Ms. Lavis:

We have received the required information regarding the purity of the Naphthalene used in the subject study. According to the attached documents from HUNTSMAN CORPORATION (formerly TEXACO CHEMICAL COMPANY) the test material was 99.9%. The notebook citation showing shipment of the material for toxicity testing, the NMR and IR spectra and GC analysis demonstrating that the material was 99,908% naphthalene are attached.

If you have any questions or require further information, please don't hesitate to contact either myself or Mrs. Peggy Galloway.

Best regards,

Wm. Ronald Landis, Ph.D.  
Regulatory Agent for RECOCHEM

pc: Ms. Lois A. Rossi, Chief, SRRD  
Mr. Ralph Carmichael, RECOCHEM

Enclosure: Naphthalene Purity Data