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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 056502. Pentachloronitrobenzene. 6(A)(2) Data. 90-Day Gavage and 21-Day Dermal Studies

Shaughnessy No. 056502
Tox. Chem. No. 640
Project No. D181572
Submission No. S423460

TO: Susan Cerrelli, PM # 73
Special Review and
Reregistration Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley
Section I, Toxicology Branch I 12/14/92
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THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I Roger Gardner 12/14/92 KB 12/15/92
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Background and Request:

Amvac Chemical Corporation has submitted a 90-day oral gavage study on pentachloronitrobenzene (PCNB) conducted with rats in response to the PCNB Registration Standard issued in 1987. In addition, Amvac has also submitted a 21-day dermal study conducted with rats on PCNB in response to a Data Call-In issued in 1990. Both of these studies were regarded as 6(A)(2) data because the thyroid effects (hypertrophy and hyperplasia of the follicular epithelium) had not been observed before in either a subchronic study or in a dermal study. The Toxicology Branch (TB-I) has been asked to review the two studies.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the submitted 90-day subchronic oral and 21-day dermal studies conducted with PCNB on rats. Both of the studies are acceptable for regulatory purposes and fulfill the guideline requirements for a 90-day subchronic oral study in rodents (82-1) and for a 21-day dermal study (82-2). Both studies are classified as Core Guideline.

The studies were classified as 6(A)(2) data. Section 6(A)(2) requires identification of data as 6(A)(2) if the data are considered to be unreasonable adverse effects. Since the Agency is already aware that this particular chemical induces thyroid effects, the Agency does not consider these data to be of significant impact that they would be classified as 6(A)(2) data. The RfD for PCNB is 0.003 based on a 2-year feeding study in the dog with a NOEL of 0.75 mg/kg/day and an uncertainty factor of 300. These new data will not affect the RfD. The following paragraphs summarize the results of the studies.

PCNB was tested in a 21-day dermal study in Crl: CDBR rats at the following dose levels: 0, 100, 300 and 1000 mg/kg/day. The NOEL was 300 mg/kg/day and the LEL was 1000 mg/kg/day based on the increased incidences of dilatation of the thyroid follicles and hypertrophy of the thyroid follicular epithelium in high dose males. There were no treatment-related effects in females.

PCNB was tested in a 90-day oral gavage study in Crl: CDBR Sprague-Dawley rats at the following dose levels: 0, 5, 10, 100 and 1000 mg/kg/day. The NOEL is 100 mg/kg/day and the LEL is 1000 mg/kg/day based on hepatocellular centrilobular hypertrophy and an increased incidence and severity of hypertrophy and hyperplasia of the follicular epithelium of the thyroid in both sexes at 1000 mg/kg/day.



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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 10/30/92
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 12/10/30
Section I, Tox. Branch (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Subchronic Oral Study in Rats (Gavage)

SHAUGHNESSY NO./TOX. CHEM. NO.: 056502 / 640

ACCESSION NO./MRID NO.: 424160-01

DP BARCODE/SUBMISSION NO.: D181572 / S423460

TEST MATERIAL: Pentachloronitrobenzene

SYNONYMS: PCNB

STUDY NUMBER(S): MRD-89-505 : 250570A

SPONSOR: Amvac Chemical Corporation, Los Angeles, CA 90023

TESTING FACILITY: Exxon Biomedical Sciences, Inc., East Millstone, NJ 08875-2350

TITLE OF REPORT: 90-Day Subchronic Oral Toxicity Study In Rats With Pentachloronitrobenzene

AUTHOR(S): R. T. Keefe

REPORT ISSUED: 6/9/92

CONCLUSION: PCNB was tested in a 90-day oral gavage study in Crl: CDBR Sprague-Dawley rats at the following dose levels: 0, 5, 10, 100 and 1000 mg/kg/day. The NOEL is 100 mg/kg/day and the LEL is 1000 mg/kg/day based on hepatocellular centrilobular hypertrophy and an increased incidence and severity of hypertrophy and hyperplasia of the follicular epithelium of the thyroid in both sexes at 1000 mg/kg/day.

Classification: Core Guideline

Testing Guideline Satisfied: 82-1

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: Pentachloronitrobenzene

Description: tan powder
Batch #(s), Other #(s): Lot No. 05318-7D Batch No. II
Purity: 98%
Source: Amvac Chemical Corporation
Vehicle: 0.2% Tween-80; Carrier: 2% Carboxymethylcellulose

2. Test Animals:

Species and Strain (sexes): Male and female Crl: CDBR Sprague-Dawley rats

Age: 7-8 weeks
Weight(s): 243 - 294 g (♂); 175 - 227 g (♀)
Source(s): Charles River Laboratories, Kingston Facility, Stoneridge, New York

3. Procedure:

a. Preparation of Test Material: The test material was suspended in the vehicle and the carrier was added to the appropriate final concentration.

Frequency of preparation: Daily

Storage conditions: The technical test material was stored at room temperature and the prepared test material dosing solutions were refrigerated.

Stability Analyses: Analyses for stability were performed during the first two weeks of the study. The analyses were conducted using HPLC with UV detection. The samples were injected in triplicate. Samples were analyzed on days 0 and 2, and on days 0, 2, 3, 4, 5 and 7.

Homogeneity Analyses: Analyses for homogeneity were also performed during the first two weeks of the study. The analyses were conducted using HPLC with UV detection. It was not stated how the samples were taken for analysis.

Concentration Analyses: Concentration analyses were conducted at least twice per week. The analyses were conducted using HPLC with UV

detection. Triplicate aliquots of each dose level were analyzed.

b. Basis For Selection of Dose Levels: The report stated that "the limit dose of 1000 mg/kg was selected as the high dose based on the Sponsor's previous experience. A range of middle doses were selected in order to obtain a clear dose response and NOAEL."

c. Animal Assignment and Dose Levels:

| Test Group | Target Dose mg/kg/day | Measured Dose mg/kg/day[a] | Total Animals male | female |
|---|---|---|---|---|
| 1 (Control) | 0 | 0 | 10 | 10 |
| 2 | 5 | 4 | 10 | 10 |
| 3 | 10 | 8.7 | 10 | 10 |
| 4 | 100 | 100 | 10 | 10 |
| 5 | 1000 | 985 | 10 | 10 |

[a]Derived from mean analytical concentrations

d. Procedures for Studies Other Than Feeding: The test material was administered by gavage in a volume of 5 mg/kg, 5 days per week for a minimum of 13 weeks. The amount of test material administered was based on the most recent body weight measurement.

e. Clinical Observations and Mortality: Animals were checked for mortality twice daily during the week and once daily on weekends and holidays. They were checked daily for clinical signs of toxicity.

f. Body Weight Determinations: Body weights were recorded during the week prior to dosing, on day 0 and weekly thereafter. They were also recorded at study termination.

g. Food and/or Water Consumption: Food consumption was measured weekly.

h. Ophthalmological Examinations: Ophthalmological examinations were conducted prior to initiation of dosing and at study termination. Both eyes of each selected rat were examined by focal illumination and indirect ophthalmoscopy. Mydriasis was produced with 1% atropine and the eyes were examined in subdued light.



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i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Clinical laboratory studies were conducted on all animals during week 7 and at terminal sacrifice. Hematology was also conducted on all animals prior to dosing. Blood samples were collected from the orbital sinus while under methoxyflurane anesthesia following an overnight fast from food.

The following CHECKED (X) parameters were examined:

| X | | X | |
|---|---|---|---|
| x | Hematocrit (HCT)* | | Mean corpuscular HGB (MCH) |
| x | Hemoglobin (HGB)* | | Mean corpuscular HGB conc. (MCHC) |
| x | Leukocyte count (WBC)* | | Mean corpuscular volume (MCV) |
| x | Erythrocyte count (RBC)* | x | Reticulocytes[a] |
| x | Platelet count* | | |
| | Total plasma protein (TP) | | |
| x | Leukocyte differential count* | | |

[a]Only if other RBC parameters were abnormal.

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

| X | Electrolytes: | X | Other: |
|---|---|---|---|
| x | Calcium* | x | Albumin* |
| x | Chloride* | x | Blood creatinine* |
| | Magnesium | x | Blood urea nitrogen* |
| x | Phosphorus* | | Cholesterol |
| x | Potassium* | | Globulins |
| x | Sodium* | x | Glucose* |
| | Enzymes: | x | Total bilirubin* |
| | Alkaline phosphatase | x | Total protein* |
| | Cholinesterase | | Triglycerides |
| | Creatinine phosphokinase* | x | Liver porphyrin |
| | Lactic acid dehydrogenase | | |
| x | Serum alanine aminotransferase (also SGPT)* | | |
| x | Serum aspartate aminotransferase (also SGOT)* | | |
| | Gamma-glutamyl transpeptidase (GGTP) | | |



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j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: Not clearly stated in report.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

k. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: All animals which died during the study.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: All high dose and controls. In addition, gross lesions, tissue masses, liver, lungs, thyroids and kidneys were examined in the low and mid-dose groups as well.



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CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines. (**) recommended by Guidelines to be preserved only if indicated by signs of toxicity or target organ involvement.

| X | | X | | X | |
|---|---|---|---|---|---|
| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
| | Tongue | x | Aorta* | x | Brain* |
| x | Salivary glands* | x | 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 | x | Adrenals* |
| x | Cecum* | xx | Kidneys* | x | Lacrimal gland** |
| x | Colon* | x | Urinary bladder* | x | Mammary gland** |
| x | Rectum* | xx | Testes* | x | Parathyroids* |
| xx | Liver* | | Epididymides | x | Thyroids* |
| | Gall bladder* | | Prostate | | Other |
| x | Pancreas* | | Seminal vesicle | x | Femur** |
| | Respiratory | xx | Ovaries | x | Skeletal muscle** |
| x | Trachea* | x | Uterus* | x | Skin |
| x | Lung* | x | Oviducts | x | All gross lesions and masses* |

1. Statistical Analyses: Comparisons were limited to within sex analysis. The equality of means were tested using an appropriate one-way analysis of variance and a test for ordered response in the dose groups. Bartlett's test was conducted first. If the variances were equal, then parametric methods were employed. Otherwise, nonparametric methods were used. The following tests were used.

Parametric: one-way ANOVA using the F distribution, Dunnett's test and a standard regression analysis for linear response in the dose groups and linear lack of fit.

Nonparametric: Kruskal-Wallis test, Dunn's Summed Rank test and Jonckheere's for monotonic trend.

The Bartlett's test was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance.



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B. RESULTS:

1. Dosing Preparation: The analytical report stated that "over five days, there was no evidence of decline of test material at the low (0.1%) and high (20%) concentrations within the accuracy of the method as indicated by the coefficient of variation of multiple infections of samples over the entire test period...Concentration verification results show the variability between aliquots and on different days to be as much as -1886% (at 0.1%) of nominal values...Averaged over the entire test period, the nominal concentrations appear to be achieved, but this reflects consistently lower than nominal concentrations during the first 55 days (approximate) of the test period and higher than nominal concentrations 35 days (approximate) of the study. Agreement at the higher concentrations was better than at the lower levels. The following table summarizes the results.

| Target | Mean ± S.D. | % C.V.[a] | Agreement % of Target | Range low | Range High |
|---|---|---|---|---|---|
| 0.1 | 0.0791 ± 0.02639 | 33.4 | 79.1 | 0.0079 | 0.149 |
| 0.2 | 0.174 ± 0.05017 | 28.8 | 87.0 | 0.055 | 0.264 |
| 2.0 | 2.00 ± 0.414 | 20.7 | 100.0 | 1.28 | 3.19 |
| 20 | 19.6 ± 4.49 | 22.9 | 98.0 | 10.6 | 36.4 |

[a]C.V. = coefficient of variation.

The stability analyses indicated that the mixtures were stable over a period of up to 7 days. However, as noted above, the results indicated that there was a considerable variability between measurements, particularly at the lower dose levels. Overall, since the means approached the nominal concentrations, these results will not affect the acceptability of the study. However, the mean measured concentrations will be the ones used for the determination of the NOEL and LEL.

2. Clinical Observations and Mortality: The report stated that there were no treatment-related deaths. There were microscopically confirmed gavage error deaths. These included 2 controls, 2 at 5 mg/kg, 1 at 10 mg/kg and 1 at 1000 mg/kg. No treatment-related clinical

signs of toxicity were observed in any of the dosed groups. In general, clinical signs were few and usually only involved 1 or 2 animals per observation. Some of the signs included alopecia, scabs, sores, changes in incisors, ocular and nasal discharge, urine and anogenital staining, dyspnea, hypothermia and emaciated appearance.

3. Body Weight Determinations: No treatment-related changes in body weights or body weight gain were observed in any of the treated groups. The body weight changes were calculated and reported weekly. Therefore, the following table summarizes body weights throughout the study.

Mean Body Weights

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Day | 0 | 28 | 63 | 91 | 0 | 28 | 63 | 91 |
| Dose mg/kg | | | | | | | | |
| 0 | 269.5 | 413.4 | 504.3 | 562.4 | 198.5 | 267.2 | 298.6 | 328.8 |
| 5 | 269.1 | 401.6 | 489.9 | 549.6 | 200.2 | 271.1 | 312.8 | 332.8 |
| 10 | 269.6 | 416.7 | 513.9 | 573.1 | 203.5 | 275.7 | 308.2 | 339.2 |
| 100 | 268.3 | 414.0 | 517.7 | 572.6 | 202.0 | 266.2 | 301.1 | 325.3 |
| 1000 | 270.8 | 424.1 | 514.9 | 573.3 | 199.7 | 267.5 | 295.1 | 321.8 |

4. Food Consumption: During weeks 2, 3, and 4, there were statistically significant differences in food consumption between the means when the treated groups were compared to controls. In addition, there was an increasing trend in food consumption from the lowest dose to the highest dose. The way the tables are presented, it is unclear as to which means were significantly different from the control. Nevertheless, these differences are not biologically significant.

5. Ophthalmological Examinations: There were no treatment-related changes. The report stated that one female from the control and 5 mg/kg groups and 2 females from the 100 mg/kg group exhibited focal retinopathy at study termination. These findings are not considered to be related to treatment.

6. Hematology: There were no consistent dose-related differences between treated and control groups. The following table summarizes several selected parameters at termination.

Selected Hematology Values at Termination

| Parameters | RBC | WBC | HCT | HGB | PLT | RBC | WBC | HCT | HGB | PLT |
|---|---|---|---|---|---|---|---|---|---|---|
| Units | x10E6 | x10E3 | % | g/dL | x10E3 | x10E6 | x10E3 | % | g/dL | x10E3 |
| Dose (mg/kg) | | | Male | | | | | Female | | |
| 0 | 8.09 | 9.8 | 47.9 | 16.1 | 600 | 7.57 | 6.4 | 47.8 | 16.2 | 707 |
| 5 | 8.09 | 9.7 | 48.8 | 16.4 | 690 | 7.62 | 6.7 | 48.1 | 16.2 | 722 |
| 10 | 7.88 | 9.0 | 47.9 | 16.1 | 655 | 7.40 | 5.9 | 47.1 | 16.1 | 648 |
| 100 | 8.03 | 11.3 | 47.4 | 16.2 | 688 | 7.20 | 6.3 | 46.2 | 15.8 | 694 |
| 1000 | 8.03 | 8.2 | 47.3 | 16.0 | 642 | 7.15 | 7.0 | 45.3 | 15.4 | 713 |

7. Clinical Chemistry: There were no treatment-related differences between the treated and the control groups. At 7 weeks in both males and females, there were several parameters that were statistically significant when compared to controls. In some cases, the differences appeared to be linear with increasing dose. Most of these cases were not significantly different when compared to the control values at termination. At 7 weeks in males, there were significant decreases in total bilirubin and in alanine aminotransferase (ALT) and significant increases in glucose, calcium and phosphorus at the high dose. With the exception of ALT, none of these were significantly different from controls at termination. The decrease in ALT is not clinically significant. Increases in ALT would be clinically significant. In females at 7 weeks there was also a significant increase in glucose and a significant decrease in ALT at the high dose. It also was stated that there was an increase in chloride, but the table did not indicate at which dose level. Again, these changes did not appear at termination except the decrease in ALT which is not clinically significant. Since the differences were not large and since they were not consistent, they are not considered to be biologically significant. There were no treatment-

related changes in mean liver porphyrin values at termination in both males and females when compared to controls at any dose level. The following table summarizes selected values at termination.

Selected Clinical Chemistry Values at Termination

| Parameter | TBILI | GLU | Ca++ | K+ | ALT | TBILI | GLU | Ca++ | K+ | ALT |
|---|---|---|---|---|---|---|---|---|---|---|
| Units | mg/dL | mg/dL | mg/dL | mmol/L | IU/L | mgdL | mg/dL | mg/dL | mmol/L | IU/L |
| Dose (mg/kg) | | | Male | | | | | Female | | |
| 0 | 0.54 | 110.6 | 9.9 | 5.36 | 39.3 | 0.53 | 99.4 | 9.7 | 140.9 | 36.8 |
| 5 | 0.41 | 98.1 | 9.7 | 4.81[a] | 36.8 | 0.50 | 98.1 | 9.9 | 142.1 | 44.7 |
| 10 | 0.46 | 101.8 | 9.7 | 5.13 | 34.4 | 0.49 | 102.2 | 10.1 | 143.5[a] | 35.3 |
| 100 | 0.49 | 105.9 | 9.7 | 4.87 | 30.2[b] | 0.51 | 103.4 | 10.1 | 141.5 | 29.4 |
| 1000 | 0.46 | 107.8 | 9.8 | 4.79[a] | 20.7[b] | 0.50 | 99.6 | 9.9 | 142.0 | 20.7[b] |

[a]Statistically significant $p<0.05$
[b]Statistically significant $p<0.01$

8. Gross Pathology: No treatment-related differences between treated and control groups were observed. Single incidences of slightly enlarged liver (control, 100 and 1000 mg/kg groups), reddened cervical lymph nodes (control, 5 and 1000 mg/kg groups), pleural and cardiac adhesions, dilated renal pelvis, distended urinary bladder with calculi and thymus discoloration were observed. The report stated that the 6 animals which succumbed prior to termination displayed postmortem findings indicative of dosing accidents: lung discoloration, lung adhesions, abnormal contents of the thoracic cavity and abnormalities of the pericardium.

9. Organ Weights: In males, there was a statistically significant increase in the mean absolute kidney weight but not in the mean relative kidney weight at the high dose when compared to the control values. In females, there was a slight, but statistically significant increase in the relative mean liver weight, but not in the absolute mean liver weight at the high dose when compared to the control values. None of the other differences in absolute and relative organ weights between the treated and control groups were statistically significant. These changes are small and are not considered to be biologically significant.

10. Histopathology: Treatment-related lesions in the liver and thyroid were observed in both sexes in the 1000 mg/kg group. These included "hypertrophied hepatocytes in the centrilobular zone with dense, homogeneous, eosinophilic cytoplasm. Changes in the thyroid included an increased incidence and severity of hypertrophy and hyperplasia of the follicular epithelium in the high dose animals". The animals which died by accident exhibited pleuritis and/or inflammation around the thymus. The following table summarizes incidences of selected microscopic lesions.

Incidence of Histomorphologic Observations

| Dose Group | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex | M | M | M | M | M | F | F | F | F | F |
| # Animals/Group | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Heart** | | | | | | | | | | |
| # Examined | 10 | 1 | 1 | 0 | 10 | 10 | 1 | 0 | 1 | 10 |
| Myocarditis, chronic, focal | 2 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Pericarditis, fibrinopurulent | 0 | 1 | 1 | 0 | 1 | 2 | 1 | 0 | 0 | 0 |
| **Kidneys** | | | | | | | | | | |
| # Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Cysts, medulla | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration, cortical tubules, focal | 0 | 3 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Liver** | | | | | | | | | | |
| # Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hypertrophy, hepatocellular, centrilobular | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 2 |
| Infiltration, mononuclear-cell, multifocal | 8 | 6 | 3 | 6 | 7 | 6 | 4 | 2 | 3 | 4 |
| **Lung** | | | | | | | | | | |
| # Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Pleuritis, fibrinopurulent | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| **Pituitary** | | | | | | | | | | |
| # Examined | 10 | 1 | 1 | 0 | 10 | 10 | 2 | 0 | 0 | 10 |
| Cysts | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| **Thymus** | | | | | | | | | | |
| # Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Inflammation, capsule, chronic and inflammation, fibrinopurulent | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 0 |

Incidence of Histomorphologic Observations

| Dose Group | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex | M | M | M | M | M | F | F | F | F | F |
| # Animals/Group | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Thyroid** | | | | | | | | | | |
| # Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hypertrophy/hyperplas. follicular epithelium | 2 | 2 | 3 | 3 | 6 | 0 | 0 | 0 | 0 | 5 |
| Vacuolation, colloid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |

11. Quality Assurance Measures: Signed Quality Assurance and Good Laboratory Statements were provided.

C. DISCUSSION: This was a well-conducted study. The study is classified as Core Guideline. There were some difficulties with the consistency of the dosing concentrations. It is not likely that these difficulties affected the outcome of the study since the overall mean doses that the treated groups were receiving were not hugely different from the nominal doses. The NOEL is 100 mg/kg/day and the LEL is 1000 mg/kg/day based on hepatocellular centrilobular hypertrophy and an increased incidence and severity of hypertrophy and hyperplasia of the follicular epithelium of the thyroid in both sexes at 1000 mg/kg/day.

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Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 12/10/92
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DATA EVALUATION RECORD

STUDY TYPE: 21-Day Dermal

SHAUGHNESSY NO./TOX. CHEM. NO.: 056502 / 640

ACCESSION NO./MRID NO.: 424160-02

DP BARCODE/SUBMISSION NO.: D181572 / S423460

TEST MATERIAL: Pentachloronitrobenzene

SYNONYMS: PCNB

STUDY NUMBER(S): Exxon Study # 150509

SPONSOR: Amvac Chemical Corporation, Los Angeles, CA 90023

TESTING FACILITY: Exxon Biomedical Sciences, Inc., East Millstone, New Jersey

TITLE OF REPORT: 21-Day Repeated Dose Dermal Toxicity Study in Rats with Pentachloronitrobenzene

AUTHOR(S): G. W. Trimmer

REPORT ISSUED: June 22, 1992

CONCLUSION: PCNB was tested in a 21-day dermal study in rats at the following dose levels: 0, 100, 300 and 1000 mg/kg/day. The NOEL was 300 mg/kg/day and the LEL was 1000 mg/kg/day based on the increased incidences of dilatation of the thyroid follicles and hypertrophy of the thyroid follicular epithelium in high dose males. There were no treatment-related effects in females.

Classification: Core Guideline

Testing Guideline Satisfied: 82-2

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: Pentachloronitrobenzene

Description: Tan powder
Batch #(s), Other #(s): Batch # II; Lot # 05318-7D
Purity: 98%
Source: Assumed Amvac Chemical Corporation
Vehicle (if applicable): Reverse osmosis water
Positive Control(s) (if applicable): N/A

2. Test Animals:

Species and Strain (sexes): Male and female Crl: CDBR rats
Age: 9 weeks (♂); 11 weeks (♀)
Weight(s): 295 - 326 g (♂); 237 - 273 g (♀)
Source(s): Charles River Laboratories, Inc., Kingston Facility; Stone Ridge, NY

3. Procedure:

a. Preparation of animals and test material and administration of test material: Twenty-four hours prior to treatment, each rat was clipped on the dorsal surface from the shoulder region to the lumber region (approximately 10% of the body surface). The skin was left intact. Each rat was reclipped every Sunday unless severe dermal irritation was noticed. The test material was weighed out according to a specified mg/kg amount for each animal. Reverse osmosis water was placed on a gauze patch at a rate of 1 ml/g test material. The test material was applied to an area of approximately 60 x 40 mm on the clipped surface of each animal, under a porous gauze dressing. It was not stated whether or not the test material was placed dry on the skin and the wet gauze patch was placed over it or the test material was applied to the wet gauze patch and then placed on the animal. The gauze dressing was secured with a non-irritating tape. Each animal was then wrapped with COBAN to prevent ingestion of the test material. Daily exposure was for 6 hours and the appropriate dose level was applied 5 days per week for a minimum of 3 weeks (16 applications over 22 days). The residual test material was removed by gently washing the exposure site with reverse osmosis water and

wiping with a paper towel. The dose levels were as follows: 0, 100, 300 and 1000 mg/kg. The controls were treated with 2 ml/kg reverse osmosis water.

b. Basis For Selection of Dose Levels: Doses were selected on the basis on results from a 7-day range-finding study. There were no signs of toxicity or dermal irritation at dose levels of 250, 500 and 1000 mg/kg/day.

c. Clinical Observations and Mortality: Each animal was observed daily for clinical signs of toxicity and twice daily for mortality (once daily on weekends and holidays). Dermal irritation was assessed on days 0, 1, 4, 7, 11, 18, 21 and 22.

d. Body Weight Determinations: Body weights were recorded prior to the study, on day 0, on days 7, 14 and 21 and at termination.

e. Food and/or Water Consumption: Food consumption was measured on days 7, 14 and 21.

f. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Blood samples were collected on day 22 from the abdominal aorta of fasted animals.

The following CHECKED (X) parameters were examined:

| X | | X | |
|---|---|---|---|
| x | Hematocrit (HCT)* | | Mean corpuscular HGB (MCH) |
| x | Hemoglobin (HGB)* | | Mean corpuscular HGB conc. (MCHC) |
| x | Leukocyte count (WBC)* | | Mean corpuscular volume (MCV) |
| x | Erythrocyte count (RBC)* | x | Reticulocytes |
| x | Platelet count* | x | Prothrombin time |
| | Total plasma protein (TP) | x | Thromboplastin time |
| x | Leukocyte differential count* | | |

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

| X | Electrolytes: | X | Other: |
|---|---|---|---|
| x | Calcium* | x | Albumin* |
| x | Chloride* | x | Blood creatinine* |
| | Magnesium | x | Blood urea nitrogen* |
| x | Phosphorus* | x | Cholesterol |
| x | Potassium* | | Globulins |
| x | Sodium* | x | Glucose* |
| | Enzymes: | x | Total bilirubin* |
| x | Alkaline phosphatase | x | Total protein* |
| | Cholinesterase | x | Triglycerides |
| | Creatinine phosphokinase | | A/G Ratio |
| | Lactic acid dehydrogenase | | |
| x | Serum alanine aminotransferase (also SGPT)* | | |
| x | Serum aspartate aminotransferase (also SGOT)* | | |
| | Gamma-glutamyl transpeptidase (GGTP) | | |

g. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: Not applicable.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

h. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: Not applicable.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: Preserved tissues were microscopically examined from the control and high dose groups, except for the thyroid gland, which was examined in the mid- and low-dose males as well. Gross lesions and tissue

masses from the mid- and low-dose groups were also microscopically examined.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

| | | | | | |
|---|---|---|---|---|---|
| X | | X | | X | |
| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
| | Tongue | | Aorta | | Brain |
| | Salivary glands | | Heart | | Periph. nerve |
| | Esophagus | | Bone marrow | | Spinal cord (3 levels) |
| | Stomach | | Lymph nodes | | Pituitary |
| | Duodenum | | Spleen | | Eyes (optic n.) |
| | Jejunum | | Thymus | | Glandular |
| | Ileum | | Urogenital | | Adrenals |
| | Cecum | xx | Kidneys* | | Lacrimal gland |
| | Colon | | Urinary bladder | | Mammary gland |
| | Rectum | xx | Testes | | Parathyroid |
| xx | Liver* | | Epididymides | x | Thyroid* |
| | Gall bladder | | Prostate | | Other |
| | Pancreas | | Seminal vesicle | | Bone |
| | Respiratory | xx | Ovaries | | Skeletal muscle |
| | Trachea | | Uterus | x | Skin* |
| | Lung | | | x | All gross lesions and masses* |

i. Statistical Analyses: Comparisons were limited in within sex analysis. Bartlett's test was performed to determine equal variance. For parametric procedures, a standard ANOVA using the F distribution was used. If significant differences among the means were indicated, Dunnett's test was used. In addition to ANOVA, a standard regression analysis for linear response in the dose groups and linear lack of fit were performed. For the nonparametric procedures, the test of equality of means was performed using the Kruskal-Wallis test. If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment group differed significantly from the control. In addition to the Kruskal-Wallis test, Jonckheere's test for monotonic trend was performed.

B. RESULTS:

1. Clinical Observations and Mortality: There were no treatment-related clinical signs of toxicity at any dose level. Intermittent signs, which began to appear

on day 7 were limited to single control, low or mid-dose females. These included alopecia, scabs and abdominal and/or urine staining. None of these signs appeared in the high dose group. There were no deaths during treatment. One high dose female died during the terminal blood collection procedure while under anesthesia. This death is not considered to be treatment-related.

2. Body Weight Determinations: No treatment-related differences in mean body weights were observed in any treated group when compared to controls. Body weight gains were not calculated in the report. The following table summarizes the mean body weights at day 0 and at day 21 and the estimated mean body gain gains for days 0 to 21. Although not statistically analyzed, it is obvious from the table that there were no dose-related differences in mean body weight gains either.

Mean Body Weights and Body Weight Gains for Days 0-21

| Dose (mg/kg) | Bodyweights (g) | | Bodyweight Gain (g) |
|---|---|---|---|
| | Day 0 | Day 21 | Day 0 - 21 |
| | **Male** | | |
| 0 | 309.2 ± 10.3[a] | 382.9 ± 31.8[b] | 73.7 |
| 100 | 312.4 ± 7.8 | 392.8 ± 11.5 | 80.4 |
| 300 | 312.6 ± 9.6 | 387.5 ± 16.0 | 74.9 |
| 1000 | 307.2 ± 8.1 | 390.0 ± 12.8 | 82.8 |
| | **Female** | | |
| 0 | 260.4 ± 12.0 | 290.0 ± 12.6 | 29.6 |
| 100 | 258.6 ± 3.9 | 284.7 ± 15.4 | 26.1 |
| 300 | 255.4 ± 14.4 | 289.3 ± 26.5 | 33.9 |
| 1000 | 260.2 ± 8.4 | 287.5 ± 13.2 | 27.3 |

[a] = standard deviation.
[b] = None of the body weights were statistically significantly different from controls at any dose level at any time period.

3. Food and/or Water Consumption: No treatment-related differences were observed in any of the treated groups when compared to controls.

4. Hematology: In males, there were no treatment-related differences in any of the treated groups when compared to controls. In females, the white blood cell count was significantly less than controls in the 300 mg/kg dose group. However, there was no dose-response. In addition, although none of the individual means for the prothrombin time in females were significantly less than the control value, there was an "ordered response to the dose levels" (i.e. there appeared to be a decreasing trend). The study authors stated that this trend was most likely due to the fact that 1 high dose rat had a significantly decreased prothrombin time which skewed the mean value downward. TB-1 agrees with this assessment. The following table summarizes some of the values provided in the report.

Mean Quantitative Hematology

| Dose mg/kg | RBC | HCT | HGB | WBC | PLT | PT | APTT |
|---|---|---|---|---|---|---|---|
| Units | x10E6 | % | g/dL | x10E3 | x10E3 | sec | sec |
| | | | **Male** | | | | |
| 0 | 7.51 | 43.0 | 15.1 | 6.8 | 1052 | 10.5 | 24.5 |
| 100 | 7.65 | 43.4 | 15.0 | 6.6 | 1178 | 11.2 | 24.9 |
| 300 | 7.45 | 42.8 | 15.0 | 6.5 | 1151 | 11.3 | 25.3 |
| 1000 | 7.37 | 42.3 | 14.7 | 5.9 | 1166 | 10.8 | 25.8 |
| | | | **Female** | | | | |
| 0 | 7.68 | 42.7 | 15.4 | 8.8 | 1212 | 9.6 | 19.0 |
| 100 | 7.75 | 42.6 | 15.3 | 6.2 | 1197 | 9.8 | 17.6 |
| 300 | 7.52 | 41.3 | 14.6 | 6.0* | 1284 | 9.4 | 18.0 |
| 1000 | 7.53 | 41.5 | 14.8 | 8.2 | 1112 | 8.6 | 19.2 |

* = Statistically significant from controls $p < 0.05$.

In summary, there were no treatment-related changes in hematological parameters in either sex at any dose level.

5. Clinical Chemistry: No treatment-related differences were observed between the control and treated groups at any dose level in either sex. There was a decreasing trend in alanine aminotransferase (ALT) in males with increasing dose. Decreases in ALT are not clinically significant. The decreasing trend is therefore not

considered to be biologically significant. There was also an increasing trend in sodium concentrations in females with increasing dose. This is not considered to be biologically significant because the increases were small. The following table summarizes some of the measured values.

Mean Serum Chemistry

| Dose (mg/ml) | Na | Glucose | ALT | BUN | Cholest. |
|---|---|---|---|---|---|
| Units | mmol/L | mg/dL | IU/L | mg/dL | mg/dL |
| | | Males | | | |
| 0 | 145.5 | 115.2 | 38.2 | 16.0 | 27.0 |
| 100 | 147.2 | 118.6 | 41.8 | 18.8 | 24.6 |
| 300 | 145.3 | 107.6 | 36.6 | 15.0 | 21.6 |
| 1000 | 146.9 | 108.8 | 28.8 | 16.2 | 26.4 |
| | | Females | | | |
| 0 | 142.1 | 110.0 | 33.8 | 16.8 | 32.4 |
| 100 | 143.6 | 117.8 | 34.0 | 16.5 | 28.3 |
| 300 | 143.2 | 113.0 | 29.2 | 21.0 | 36.2 |
| 1000 | 144.5 | 103.2 | 30.8 | 15.0 | 33.8 |

6. Dermal Evaluations: All of the erythema and edema scores were zero at all dose levels. There was some tape irritation around the dose site in some female animals at all dose levels, including controls. This was observed starting at day 11 in 1 of 4 females which experienced this irritation in the high dose group, at day 18 in 1/4 females in the low dose group and at day 21 in 1/4 females in the controls and in 1/3 females in the mid-dose group. The slight tape irritation around the dose site is not considered to be treatment-related.

7. Gross Pathology: There were no treatment-related effects at any dose level when compared to controls. The following list of observations were noted.

| Dose Level (mg/kg) | 0 | | 100 | | 300 | | 1000 | |
|---|---|---|---|---|---|---|---|---|
| Observation | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Liver: discoloration | 3 | 0 | 3 | 0 | 1 | 1 | 1 | 0 |
| Lymph nodes: enlarged | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Kidney: dilated pelvis | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Urinary bladder: distended; mucosa thickened; hard white objects within. | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Urinary bladder: vascularized | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Ovary: large; surrounded by fluid-filled sac | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Uterus: distended | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| Cervix: thickened | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

8. Organ Weights: There were no statistically significant differences in either mean absolute or mean relative organ weights at any dose level when compared to controls.

9. Histopathology: In high dose males, dilatation of the thyroid follicles was observed in 3/5 animals and hypertrophy of the thyroid follicular epithelium was observed in 4/5 animals. These changes were not observed in any of the other dose groups, including controls or in any females. No other treatment-related effects were observed in either males or females. The following table summarizes selected microscopic observations.

Incidence of Selected Microscopic Lesions in 21-Day Dermal Study

| Dose Group (mg/kg) | 0 | 100 | 300 | 1000 | 0 | 100 | 300 | 1000 |
|---|---|---|---|---|---|---|---|---|
| Sex | ♂ | ♂ | ♂ | ♂ | ♀ | ♀ | ♀ | ♀ |
| Number of Animals/Group | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| **Treated Skin** | | | | | | | | |
| Hyperplasia, sebaceous glands | 1 | -[a] | - | 2 | 0 | - | - | 0 |
| Hyperplasia/hyperkeratosis, epidermis | 4 | - | - | 5 | 4 | - | - | 5 |
| **Thyroid** | | | | | | | | |
| Dilatation, follicles | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Hypertrophy, follicular epithelium | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| **Kidneys** | | | | | | | | |
| Mineralization, medulla, focal | 0 | - | - | 0 | 5 | - | 0[b] | 4 |
| Dilatation, pelvis, bilateral | 0 | - | - | 0 | 0 | - | 1[b] | 0 |
| Dilatation, pelvis, unilateral | 0 | - | - | 0 | 1 | - | 0 | 0 |
| **Liver** | | | | | | | | |
| Infiltration, mononuclear-cell, multifocal | 4 | 2[c] | 1[b] | 5 | 3 | - | 0[b] | 4 |
| Necrosis, focal | 2 | 3[c] | 1[b] | 1 | 0 | - | 1[b] | 0 |
| **Urinary Bladder** | | | | | | | | |
| Cystitis, hyperplastic | - | 1[b] | - | - | - | - | 1[b] | - |
| **Ovaries** | | | | | | | | |
| Cyst(s), intraovarian | - | - | - | - | 0 | - | 1[b] | 0 |

[a]"-": This tissue/organ was not examined at this dose level.
[b]This tissue/organ was examined in one animal at this dose level.
[c]This tissue/organ was examined in three animals at this dose level.

10. Quality Assurance Measures: Signed Quality Assurance and Good Laboratory Practice Statements were provided.

C.. DISCUSSION: This was a well-conducted study. The NOEL is 300 mg/kg/day and the LEL is 1000 mg/kg/day based on the increased incidences of dilatation of the thyroid follicles and hypertrophy of the thyroid follicular epithelium in males. There were no treatment-related effects in females. The study is classified as Core Guideline.