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

007547

OCT 13 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Technical Dicloran - 21-Day Dermal Study
Rabbits

TO: L. Schnaubelt/J. Mitchell
Product Manager (21)
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. 10/5/89
Toxicology Branch II, Section II
Health Effects Division (H7509C)

THRU: K. Clark Swentzel 10/6/89
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Health Effects Division (H7509C)

and

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Registrant: NOR-AM Chemical Co.
Chemical: 2,6-dichloro-4-nitroaniline
Synonym: DCNA, Botran™, Dicloran
Project: Follow up to # 8-0740
Caswell No.: 311
Record No.: 222449
Identifying No.: N/A
MRID No.: N/A
Action Requested: This is a follow up to a previous memo dated June 13, 1983 from M. Jones regarding reconsideration of a Section 18.

Comment: This is a follow-up to a previous action. A 21-day dermal study in rabbits has been reviewed, and the DER is attached.

The NOEL for systemic toxicity can be set at 120 mg/kg, and the LEL can be set at 1200 mg/kg, based on increased adrenal weights. Slight dermal irritation at the site of application was observed at the mid- and high-dose levels.

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Reviewed by: Linda L. Taylor, Ph.D. 10/4/89
Section II, Tox. Branch II (H7509C)
Secondary reviewer: K. Clark Swentzel 10/6/89
Head Section II, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity - Rabbits TOX. CHEM. NO.: 311

MRID NO.: 405551-01

TEST MATERIAL: Dicloran technical

SYNONYMS: 2,6-dichloro-4-nitroaniline; DCNA; Botran™

STUDY NUMBER: TOX 86095; SMS 60/871292

SPONSOR: NOR-AM Chemical Company

TESTING FACILITY: Huntingdon Research Centre
Huntingdon, Cambridgeshire ENGLAND

TITLE OF REPORT: T106 TECHNICAL DICLORAN: Twenty-one Day Dermal Toxicity Study in Rabbits

AUTHORS: P.H. Elliot and C. Smith

REPORT ISSUED: January 22, 1988

CONCLUSIONS: Rabbits dermally exposed to technical dicloran for 21-days at dose levels of 12, 120, and 1200 mg/kg/day displayed slight dermal irritation at the site of application at the mid- and high-dose levels. The NOEL for systemic toxicity can be set at 120 mg/kg, and the LEL can be set at 1200 mg/kg, based on increased adrenal weights.

Classification: Core minimum.

QUALITY ASSURANCE: A quality assurance statement was provided.

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A. MATERIALS:

1. Test compound: technical Dicloran; Description: yellow crystalline solid; Batch No.: CR 20642/3; Purity: 96.2-97.5%; Stability: shown to be stable under the conditions of the study.

2. Test animal: Species: Rabbit; Strain: New Zealand white; Age: 10-12 weeks on arrival; Weight: 2.2-2.5 kg; Source: Interfauna U.K. Ltd., Wyton, Huntingdon, Cambridgeshire.

3. Statistics: Data were analyzed as described on pages 21-22 of the final report (copy attached).

B. STUDY DESIGN:

1. Methodology

Rabbits were assigned randomly (using a computer program-body weight) to the following test groups.

| Test Group | Test Material (mg/kg/day) | Males | Females |
|---|---|---|---|
| 1 | 0 (distilled water) | 5 | 5 |
| 2 | 12 | 5 | 5 |
| 3 | 120 | 5 | 5 |
| 4 | 1200 | 5 | 5 |

Each animal was caged individually and had free access to water and food (SQC Rabbit Diet). The hair was clipped from the mid-dorsal region of each animal (exposing approximately 10% of the total body surface area) about 24 hours prior to initial exposure, and as needed thereafter. The test material was moistened with water, spread evenly over the treatment area, and the treatment site covered with an impervious bandage (gauze covered with "Elastoplast" elastic adhesive dressing backed with impervious "Sleek" plaster). The test material remained in place for approximately 6 hours each day for 21 days. Following exposure, the dressings were removed, the treated skin was washed with warm water and gently patted dry, and perspex "Elizabethan" collars were put on each animal between exposures to minimize ingestion of the test material. Control animals were treated similarly. Dose levels were based on the most recent body weight of the animal.

2. Observations

All rabbits were examined daily for signs of ill health, behavioral changes, or other signs of toxicosis, and twice daily for morbidity and mortality. Prior to each application and daily, the skin of each animal was graded according to the Draize system.

All animals were weighed on the first day of treatment, weekly thereafter, and immediately prior to terminal sacrifice. Food consumption was measured at weekly intervals.

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RESULTS

Survival and Clinical Signs

No deaths occurred during the study. Yellow staining of the untreated fur and extremities was noted in the mid- and high-dose groups from Day 4 on, which was considered to be from contact with the test material. No other signs were observed that were associated with treatment.

Body Weight and Food Consumption

No differences were reported for either body weight or food consumption among the groups.

Dermal Irritation

Two rabbits (one/sex) in the low dose group displayed slight, transient erythema. All of the mid-dose rabbits displayed slight erythema (most displaying this reaction by the second week), and slight edema was observed in four males and three females of this group during the third week. Both reactions tended to persist to study termination.

At the high-dose level, slight erythema was observed during the second week of exposure, but by days 12 or 16, yellow staining of the treated skin precluded the assessment of erythema for the remainder of the study. Slight edema was observed on day 8 in one high-dose female, and two high-dose males and all high-dose females displayed edema from days 13 to 17, which persisted to study termination.

3. Clinical Pathology

Blood was collected from each animal at necropsy (week 3) for hematology and clinical analyses following an overnight fast. The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT) | X | Leukocyte differential count |
| X | Hemoglobin (HGB) | | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC) | X | Mean corpuscular HGB conc.(MCHC) |
| X | Erythrocyte count (RBC) | X | Mean corpuscular volume (MCV) |
| X | Platelet count | | Nucleated red blood cell count |
| X | Heinz bodies (HzB) | X | Cellular morphology |
| X | Methemoglobin(MetHb) | X | Reticulocyte count |
| | | X | Thrombotest (TT) |

RESULTS

With two exceptions, the hematological parameters were comparable among the groups. Slightly lower thrombotest times were recorded for the treated animals compared to controls, with statistical significance being achieved in the males. The magnitude of the shift was small, and the individual values were said to be within the expected range for this

parameter, although no historical control data were presented. Additionally, statistically significantly lower methemoglobin levels were recorded for all treated females, but the increase was not dose-related.

| | C | L | M | H | C | L | M | H |
|---|---|---|---|---|---|---|---|---|
| Thrombotest time (s) | 22 | 19* | 20* | 20* | 20 | 19 | 19 | 19 |
| Met Hb (% Hb) | 0.72 | 0.53 | 0.69 | 0.67 | 0.95 | 0.44† | 0.57† | 0.42† |

*p<0.05 in comparison with controls using Williams' test
†p<0.01 in comparison with controls using Williams' test

b. Clinical Chemistry

| | Electrolytes: | | Other: |
|---|---|---|---|
| X | Calcium | X | Albumin |
| X | Chloride | X | Blood creatinine |
| | Magnesium | X | Blood urea nitrogen |
| X | Phosphorous | X | Cholesterol |
| X | Potassium | X | Globulins |
| X | Sodium | X | Glucose |
| | Enzymes | X | Total Bilirubin |
| X | Alkaline phosphatase | X | Total Serum Protein |
| | Cholinesterase | | Triglycerides |
| | Creatinine phosphokinase | | Serum protein electrophoresis |
| | Lactic acid dehydrogenase | X | Albumin/Globulin ratio |
| X | Serum alanine aminotransferase (also SGPT) | | |
| X | Serum aspartate aminotransferase (also SGOT) | | |
| | gamma glutamyl transferase | | |
| | glutamate dehydrogenase | | |

RESULTS

Higher globulin levels ($p<0.05$) were recorded in the high-dose males, which resulted in significantly lower ($p<0.05$) albumin/globulin ratios. These shifts were reversed in the female rabbits, and the individual values for the males were said to be within the expected range for this parameter (again, no historical control data were presented). These were not considered to be related to treatment. Since only terminal measurements were performed, any apparent effect cannot be ascribed to treatment.

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | C | L | M | H | C | L | M | H |
| Globulin | 1.9 | 2.1 | 1.9 | 2.3* | 2.1 | 2.0 | 2.0 | 1.7 |
| A/G | 2.09 | 1.99 | 2.00 | 1.70* | 1.85 | 1.95 | 1.97 | 2.31* |

*p<0.05 in comparison with controls using the Williams' test

4. Gross Pathology

All animals were sacrificed (after overnight food fast) and were subjected to gross pathological examination.

The liver, kidneys, adrenals, spleen, ovaries, and testes (with epididymides) were disected free of fat and weighed. The following tissues were preserved for histological examination from all control and high-dose rabbits:

| | |
|---|---|
| kidneys | skin (treated and untreated) |
| liver | and other macroscopically abnormal tissue |
| spleen | |

RESULTS

Adrenal weights were significantly greater ($p<0.05$) in the high-dose males compared to control, and the high-dose females also displayed heavier adrenals compared to their respective controls, but a $p<0.05$ was not attained. All other organ weights were comparable among the groups.

| | Males | Females |
|---|---|---|
| Control | 191 | 190 |
| Low | 194 | 204 |
| Mid | 213 | 180 |
| High | 260* | 211 |

* $p<0.05$ in comparison with control using Williams' test

Several mid- and high-dose animals displayed general yellow staining of the fur at post mortem. No other differences were noted.

5. Histopathology

The following additional organs and tissues were preserved (apparently from control and high-dose animals), but were not processed further.

| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta | X | Brain |
| X | Salivary glands | X | Heart | X | Periph. nerve (sciatic) |
| X | Esophagus | X | Bone marrow | | Spinal cord |
| X | Stomach | X | Lymph nodes* | X | Pituitary |
| X | Duodenum | X | Spleen† | X | Eyes |
| X | Jejunum | X | Thymus | | Glandular |
| X | Ileum | | Urogenital | X | Adrenals |
| X | Cecum | X | Kidneys† | | Lacrimal gland (Harderian) |
| X | Colon | X | Urinary bladder | X | Mammary gland |
| | Rectum | X | Testes | X | Parathyroids |
| X | Liver† | X | Epididymides | X | Thyroids |
| X | Gall bladder | X | Prostate | | Other |
| X | Pancreas | | Seminal vesicle | X | Bone (sternum) |
| | Respiratory | X | Ovaries | X | Skeletal muscle |
| X | Trachea | X | Uterus | X | Skin(treated and untreated)† |
| X | Lung | | Cervix | X | All gross lesions |
| | Nose | X | Vagina | | and massest |
| X | Pharynx | | Oviduct | | Head |
| X | Larynx | | | | Coagulating gland |
| | | | | | Mediastinal/mesenteric tissue |

* cervical and mesenteric
†intermediate and low dose groups

RESULTS

No differences were noted among the groups that could be related to test material exposure.

C. CONCLUSION:

The NOEL for systemic toxicity can be set at 120 mg/kg, and the LEL can be set at 1200 mg/kg, based on increased adrenal weights. Slight dermal irritation at the site of application was observed at the mid- and high-dose levels.

Microscopic examination

Fixed tissue samples were embedded in paraffin wax (m.p. 56°C), sections cut at 4µm stained with haematoxylin and eosin.

Microscopic examinations were carried out for tissues listed under "Terminal studies" from all rabbits of the control and high dosage groups.

Statistical analyses

All statistical analyses were carried out separately for males and females.

Food consumption data were analysed using cumulative totals. Bodyweight data were analysed using weight gains.

The following sequence of statistical tests was used for food consumption, bodyweight, organ weight and clinical pathology data:

(i) If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of values different from the mode was analysed by appropriate methods. Otherwise:

(ii) Bartlett's test (1) was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

(iii) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.

(iv) Analyses of variance were followed by a Student's 't' test and Williams' test (4) for a dose-related response, although the more appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test, (3)).

For organ weight data, where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. The final bodyweight was used as covariate in an attempt to allow for differences in bodyweight which might have influenced the organ weights.

References

1. Bartlett, M.S., (1937), Proc. Roy. Soc. 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. Shirley, E., (1977), Biometrics, 33 : 386-389.

4. Williams, D.A., (1971/2), Biometrics, 27 : 103-117 and 28 : 519-531.

Good laboratory practice

The study was conducted in accordance with the following principles of Good Laboratory Practice:

OECD Good Laboratory Practice Principles, ISBN 92-64-12367-9, Paris 1982.

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983.

Quality assurance review

The Department of Quality Assurance conducted inspections of the various phases of the study as required by the above Good Laboratory Practice principles. The dates on which the findings of these inspections were reported to the Study Director and to HRC Management are specified in this report.

This report was reviewed by HRC Department of Quality Assurance, comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the appendices of the report.