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ACUTE INHALATION TOXICITY STUDY OF 4-(N-ETHYL-N-2-HYDROXYETHYL)-2-METHYLPHENYLENEDIAMINE SULFATE (CD-4) IN THE RAT WITH ATTACHMENTS AND COVER LETTER DATED 052588		
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Environmental Protection Agency
401 M Street S.W.
Washington, D.C. 20460
Attention: 8(e) Coordinator

8EHQ-0688-0575 SUP.
PDCN: 88-860000032
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000584168W

Dear Sir or Madam:

This letter with enclosure provides supplemental information on a previous TSCA section 8(e) notification on 4-(N-ethyl-N-2-hydroxyethyl)-2-methylphenylenediamine sulfate, otherwise known as CD-4. The notification has been identified by EPA control number 8EHQ-1185-0575.

Enclosed please find a copy of an acute inhalation LC50 study in rats exposed to CD-4.

The original submission was based on an oral LD50 study in rats that showed kidney toxicity at low dose levels. The acute inhalation LC50 study was conducted to investigate whether or not kidney toxicity would be seen after inhalation exposure. No kidney or other target organ effects were observed at the highest practicable exposure level of 164 mg/m³. These results indicate that inhalation exposure to CD-4 presents less of a hazard than comparable oral doses. If you have any technical questions or would like to discuss the report, please call William L. Hart, a toxicologist in the Health and Environment Laboratories, at (716) 722-5991.

Sincerely,

R. Hays Bell, Ph.D., Director
Health and Environment Laboratories

WLH
Enc

cc: Dr. J. Loranger, OTS, EPA

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Acute Inhalation Toxicity Study of 4-(N-Ethyl-N-2-hydroxyethyl)-
2-methylphenylenediamine Sulfate (CD-4) in the Rat

Acc. No. 904984 HAEL No. 87-0098

Report prepared by:

Gary Katz, Ph.D.

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, NY 14650

Date of Study Completion: May 17, 1988

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Acute Inhalation Toxicity Study of 4-(N-Ethyl-N-2-hydroxyethyl)-
2-methylphenylenediamine Sulfate (CD-4) in the Rat

Acc No. 904984

HAEL No. 87-0098

Abstract

Groups of five male and five female rats were exposed to a particulate atmosphere of 0 or 164 mg/m³ CD-4 for six hours. No mortality was observed and body weight gains of animals exposed to CD-4 were comparable to control values. Clinical signs associated with exposure included deposition of CD-4 on the fur, porphyrin tears and porphyrin nasal discharge in both sexes and brown colored urine in males only. The nose and eye effects resolved within 48 hours after exposure and were probably a response to the irritative property of CD-4 dust. The discolored urine was probably the result of excretion of oxidized CD-4 or CD-4 metabolites in the urine as has been previously observed. Absolute and relative heart and kidney weights were comparable to control values. There were no compound-related changes detected at necropsy and histopathologic examination of the heart and kidneys revealed no compound-related effects.

Previous studies have shown that the no-effect level for toxicity by the oral route following administration of a single dose was about 3 mg/kg for males and 6 mg/kg for females. The estimated inhaled dose, based on 50% being within the respirable range i.e., 82 mg/m³, was equivalent to an oral dose of 12.1 mg/kg for males and 15.3 mg/kg for females both of which are within the range of doses producing kidney toxicity when CD-4 is given orally as a single dose. Therefore, the data suggest that a CD-4 inhalation exposure is likely to be less nephrotoxic than an equivalent oral dose given as a bolus.

The no-effect level for this study was 164 mg/m³ which was equivalent to 82 mg/m³ of respirable particulate CD-4.

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Acute Inhalation Toxicity Study of 4-(N-Ethyl-N-2-hydroxyethyl)-
2-methylphenylenediamine Sulfate (CD-4) in the Rat

Acc. No. 904984 HAEL No. 87-0098

Purpose

The purpose of this study was to determine the acute inhalation toxicity of particulate CD-4 in the rat following a single six hour exposure.

Sponsor

Eastman Kodak Company, Rochester, NY 14650

Testing Facility

Toxicological Sciences Laboratory, Health and Environment Laboratories,
B-320 Kodak Park, Eastman Kodak Company, Rochester, NY 14650.

Date of Study Initiation

December 7, 1987.

Test Procedures

This study was conducted by procedures comparable to the Toxic Substances Control Act Test Guidelines (Environmental Protection Agency, 50 FR 39252, September 27, 1985 and revised guidelines 52 FR 19056, May 20, 1987); Guidelines for Testing of Chemicals: TG-403, Acute Inhalation Toxicity (Organisation for Economic Cooperation and Development, May, 1981); and Annex V.B.2, Acute Inhalation Toxicity (European Economic Community, September, 1984).

Test Article Characterization

Name: 4-(N-Ethyl-N-2-hydroxyethyl)-2-methylphenylenediamine Sulfate
Alternate Name: CD-4
SRID No: 75013
Acc. No: 904984
HAEL No: 87-0098
Experiment No: 870098I2
Empirical formula: $C_{11}H_{20}N_2O_5S$
Molecular weight: 292.35

A sample of the CD-4 that was submitted to the Chemical Quality Services Division (CQSD), Kodak Park, for analysis by high pressure liquid chromatography was 99.9% pure. The CD-4 was used in the inhalation study as received without the addition of a vehicle.

Exposure Conditions

A single six-hour inhalation exposure study of a particulate atmosphere of CD-4 was conducted in a 420 L stainless steel and glass inhalation chamber. The target concentration was 250 mg/m³. The chamber was maintained at a slight negative pressure relative to room air, and at five air changes per hour in order to increase the concentration of particulate CD-4 suspended in chamber air. Male and female rats were exposed simultaneously in the same inhalation chamber.

Animals were placed in wire-mesh cylinders (restrainers) designed to permit movement but maintain the animals' orientation towards the center of the inhalation chamber (see Appendix - Figure 2). The restrainers (Appendix Figure 3) were used to limit exposure to only the head and to restrict grooming during exposure thereby preventing ingestion of CD-4. In addition, the perimeters of the restrainers were covered with a strip of bedding paper (Desorb bedding paper; Shepherd Specialty Papers, Inc., Kalamazoo, Michigan) such that only the head extended beyond the restrainer covering, reducing deposition of CD-4 on the fur which could be ingested during post-exposure grooming. Control animals were treated in the identical manner except that exposure was to air only.

An atmosphere of particulate CD-4 was produced by dispersing the test material into the turret of the inhalation chamber using a Wright dust feed mechanism. The Wright dust feed mechanism was supplied with metered, dried, oil-free compressed air (Kodak Park Utilities Division). A diagram of the 420 L Inhalation chamber outfitted with the Wright dust feed mechanism and animal restrainers is shown in Appendix - Figure 1.

Chamber particulate concentrations were determined six times during the exposure by gravimetric analysis of filter paper samples. Chamber temperature and relative humidity were recorded twice per hour, and the nominal aerosol concentration was calculated.

The particle size of a sample of CD-4 was determined by GQSD using a Leeds and Northrup standard range analyzer. Photomicrographs of the sample (400x) were also taken. The particle size distribution of airborne particulate CD-4 in the inhalation chamber was measured once during the exposure using an Andersen personnel particle sizing sampler (Andersen 2000, Inc., Atlanta, GA). Gravimetric analyses of the sampler collection stages were used to calculate the cumulative percent of particles by mass less than 4.7 µm in diameter, providing an estimate of the respirable fraction of the particulate atmosphere to which the animals were exposed.

Chamber aerosol distribution was determined in a pre-study test, by measuring the particulate concentration once from each of three different cage positions and three times from a fixed reference point within the inhalation chamber B-04 (Appendix Figure 2). There were no significant differences between

the mean concentrations of the reference position and the three cage positions (Appendix Table I).

A complete description of CD-4 dust generation, sampling, and analysis is presented in the appendix.

Test Animals

Rats were chosen for this study, because they are a common representative species for toxicity studies. Rats [CRL:CD[®](SD)BR] obtained from Charles River Laboratories, Inc., (Wilmington, MA.) were selected by clinical examination, and according to body weight using the Automated Animal Toxicology System GULL program. Ten male and ten female rats weighing (mean \pm SD) 165.3 \pm 4.6 g or 164.7 \pm 4.5 g, respectively, were selected for this study.

Housing

During the isolation period (five days), rats were group housed, separated by sex in suspended stainless steel cages in Room 1054 of the vivarium. The temperature and relative humidity was 70-74°F (21.1-23.3°C) and 50% respectively. The animals were released from isolation in good health and singly housed in multi-compartmented stainless steel mesh cages in room 1028 (chamber C-08) during non-exposure periods. Male and female rats were housed on the same rack. The temperature and relative humidity was 69-74°F (20.6-23.3°C) and 55-81% respectively. A 12 hour (6 a.m. to 6 p.m.) photoperiod was maintained. Absorbent paper under the cages was changed three times per week. Cages and racks were washed once per week.

Feed and Water

Certified Rodent Diet (Agway[®] Prolab[™] RMH 3000; pelleted) and water, were available ad libitum only during nonexposure periods. Water was available through an automatic watering system. The source of the water was the Monroe County Water Authority. No known contaminants were expected to be present in feed or water, which would interfere with the outcome of this study. Analyses of feed and results of quarterly analyses of water are maintained on file with the testing laboratory.

Identification

All rats were uniquely identified by metal ear tags.

Randomization

All randomization and culling was achieved by computer-generated lists using the Automated Animal Toxicology System.

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Body Weight

Body weight was measured on Days 0, 3, 7, 10, and 14. Body weight on Day 14 was compared to initial body weight on Day 0 as a measure of growth.

Clinical Observations

Rats visible through chamber windows were observed for mortality during exposure. At about 8 a.m. and 3 p.m. on each workday during the study, each rat was removed from its cage by a trained technician and observed for clinical signs of toxicity. Observations included, but were not limited to, examination of the hair, skin, eyes, respiratory system, circulatory system motor activity, feces, urine, and behavior pattern.

Pathology Examinations

Rats were fasted for about 16 hours prior to necropsy on Day 15. Animals were anesthetized with CO₂ and exsanguinated by severing the posterior vena cava. The following tissues were examined at necropsy in all animals: nasal passages, trachea, lungs, heart, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pancreas, liver, salivary glands, kidneys, urinary bladder, pituitary gland, adrenal glands, thyroid glands, parathyroid glands, thymus, spleen, mesenteric lymph nodes, bone marrow (femoral), brain, eyes, skin, plus testes, epididymides, and accessory glands in males, and ovaries, vagina, uterus, and Fallopian tubes in females. The kidneys and heart from each animal were weighed and then fixed in 10% buffered formalin for microscopic examination.

Data Storage

The final report, data sheets, and all non-perishable raw data were stored in the HAEL arch ves.

Statistical Procedures

All continuous mean data were evaluated using the following computer-generated statistical tests: one-way analysis of variance (ANOVA, $P \leq 0.05$), Bartlett's test ($P \leq 0.01$), and Duncan's multiple range test ($P \leq 0.05$). A two-tail "t" test ($P \leq 0.05$), assuming equal variances, was used to evaluate particulate distribution within the inhalation chamber.

GLP Statement

This study was conducted according to Good Laboratory Practice for Nonclinical Laboratory Studies as promulgated by the Food and Drug Administration, 21 CFR Part 58, December 22, 1978, and revised 21 CFR Part 58, September 4, 1987, Environmental Protection Agency Good Laboratory Practice Standards 40 CFR Part 792, November 29, 1983; and OECD Principles of Good Laboratory Practice.

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Project Participants

Study Director	G. Katz, Ph.D.
Study Technicians	L. Sakal, B.S.
	F. Renner, Jr., B.A.
Pathologist	M. Vlahovic, D.V.M., Ph.D.
Laboratory Animal Medicine	G. Hankinson, D.V.M., M.S.
Analytic Chemist	I. Allen, Ph D.

Results

Exposure Conditions

A target concentration of 250 mg/m³ was chosen based on the maximum concentration which could be generated for this particular chemical using the Wright dust feed mechanism. However, the animals were actually exposed to a weighted mean CD-4 dust concentration of 164 mg/m³ based on gravimetric analysis of filter paper samples. The mean was calculated from the individual exposure concentrations weighted for exposure duration. The chamber concentrations of CD-4 were approximately 95 mg/m³ during the first 2.5 hours of exposure, approximately 39 mg/m³ for the following 0.5 hour and from 214 to 266 mg/m³ for the final 3 hours. Chamber concentration decreased when the pressure, under which the CD-4 in the generator was packed, began to decrease. To achieve a full six hours of exposure, the exposure time was extended approximately 0.5 hour (to 6.5 hours) to make up for the time that exposure was stopped to repack the Wright dust feed mechanism cylinder.

The nominal concentration was substantially higher than the mean analytic concentration. The nominal concentration (1890 mg/m³) which is a measure of the generating system output, is generally higher than the suspended fraction of inhalable particulate material measured (analytic concentration). This is due to a number of factors including adsorption of test material to chamber, cage, and animal surfaces, and to the particle size output of generator which includes particles too large to remain suspended in air.

A pre-study analysis of the particle size of the compound indicated that only about 10% of the compound was less than 5.5 μm in diameter (mass diameter). Photomicrographs of the sample showed particles to be irregular in shape and with a broad size distribution. Analyses of particle size distribution from within the inhalation chamber, under dynamic conditions of airflow, indicated that 46% of the sampled particulates had an aerodynamic mass diameter less than 4.7 μm (Appendix Table 2).

Overall chamber temperature and relative humidity varied from 66-71°F (18.9 to 21.7°C) and 34 to 66%, respectively. A summary of chamber exposure conditions is presented in Table I.

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Mortality

No animals died during the study.

Body Weight

Individual and mean body weights are presented in Table II for males and females. All males and females exposed to 164 mg/m³ exhibited weight gains comparable to that of control values.

Clinical Signs

During exposure two males exhibited a porphyrin nasal discharge and one female showed hyperventilation.

Post-exposure clinical signs are summarized in Tables III and IV for males and females, respectively. Five males exposed to 164 mg/m³ exhibited porphyrin tears and porphyrin nasal discharge, and on the following day, brown discoloration of the urine. Three of the five animals exhibited brown or black discolored facial hair. One of these also showed black or brown discolored hair of the arm and brown discoloration of the tail. One of these males and a fourth male exhibited brown discoloration of abdominal hair. The fourth male also showed brown discoloration of the scrotum. In addition, the fourth and a fifth male exhibited brown discoloration of the tail. Similar effects were not seen in control animals.

Four of the five females exposed to 164 mg/m³ exhibited porphyrin tears and all five females showed a porphyrin nasal discharge. All five females also exhibited brown or black discolored abdominal hair and brown or black discolored tails. One of these also showed a black or brown discoloration of the hair of the face and arm. One other animal had brown discoloration of the hair on the arm. Two females in the control group exhibited porphyrin tears; one of which showed a porphyrin nasal discharge as well.

The discoloration of the urine and the fur were clearly related to exposure to CD-4. The urine discoloration was probably the result of excretion of oxidized CD-4 or a CD-4 metabolite. No signs of toxicity accompanied these changes.

The porphyrin nasal and eye discharges, which were probably a response to the irritative properties of CD-4 dust deposited in the nose and eyes, disappeared within 24 to 48 hours after exposure. Discoloration of fur and hair in general remained to the end of the study. Porphyrin discoloration around the eyes and nose is also occasionally seen in normal rats. No clinical signs of toxicity were evident.

The dust covers over the restrainers in which the animals were housed during exposure were not adequate to prevent deposition of CD-4 on the fur of the animals, or limit exposure to the head only. In fact, the particulate

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atmosphere was readily visible within those restrainers close to the inhalation chamber windows. In addition, five control and four CD-4 exposed animals were observed to reorient themselves within the restrainers such that they were no longer facing the center of the chamber. The frequency of the reorientations or the total number of animals involved is unknown, but inhalation exposure was probably not affected since the dust atmosphere was observed within the restrainers as well. To reduce the potential ingestion component associated with grooming, the entire fur coat of each rat was vacuumed following exposure.

Pathology Examinations

Except for minor to moderate brown discoloration of the hair and skin there were no compound-related changes detected on necropsy examination of the males and females. Absolute and relative kidney and heart weights were comparable to control values for both sexes. There were no-compound related changes detected on histopathologic examination of the kidneys or hearts. The pathologist's report is appended.

Discussion

The respirable fraction of CD-4 as received from the sponsor was relatively small. Only 10% of the CD-4 was less than 5.5 µm in diameter. However, the methodology used to create an airborne exposure to CD-4 also increased the respirable fraction to about 50%. Previous studies by the oral route have demonstrated that the no-effect level for kidney toxicity following the administration of single doses of CD-4 in rats is about 3 mg/kg in males and 6 mg/kg in females.

In the present study, rats were exposed to 164 mg/m³ CD-4 for 6 hours. This concentration may be translated into a mg/kg dose by the following equation.

$$D = \frac{C \cdot R \cdot F \cdot T \cdot 10^3}{W \cdot 10^6}$$

- Where:
- D = equivalent oral dose (mg/kg)
- C = exposure concentration (164 mg/m³)
- R = minute volume (100 cc/min)
- F = respirable dust fraction (50%)
- T = exposure duration (360 min)
- W = mean body weight (males: 231g; females: 193g)
- 10³ = conversion factor for g to kg
- 10⁶ = conversion factor mL to m³

It was also assumed that 100% of the inhaled CD-4 was deposited in the lungs.

The resultant inhaled dose was 12.8 mg/kg for males and 15.3 mg/kg for females, and is within the range of doses producing kidney toxicity in previous single dose oral studies. However, the total dose to the animal was probably somewhat higher since the restrainer covers failed to