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TWO-WEEK ORAL TOXICITY STUDY OF 4-(N-ETHYL-N-2-HYDROXYETHYL)-2-METHYLPHENYLENE DIAMINE SULFATE WITH COVER LETTER DATED 111787		
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8(e) Coordinator
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Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

CONTAINS NO CBI

Dear Sir or Madam:

Enclosed please find a copy of a two-week oral toxicity study conducted on 2-((4-amino-3-methylphenyl)ethylamino)ethanol sulfate (EPA Document Control Number 8EHQ-1185-0575). This report is being submitted as a follow-up to our submission of November 11, 1985 on this compound. If you have any questions, please contact me at the address below.

Sincerely,

R. Hays Bell

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

RHB:JAF
Enc.

CONTAINS NO CBI

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TX-87-141

TWO-WEEK ORAL TOXICITY STUDY OF
4-(N-ETHYL-N-2-HYDROXYETHYL)-2-METHYLPHENYLENE DIAMINE SULFATE (CD-4)

HAEL NO. 86-0039 ACC. NO. 904984

BY GARY V. KATZ, PH.D.

TOXICOLOGICAL SCIENCES SECTION
HEALTH AND ENVIRONMENT LABORATORIES
EASTMAN KODAK COMPANY
ROCHESTER, NY 14650

DATE OF STUDY COMPLETION: November 9, 1987

TWO-WEEK ORAL TOXICITY STUDY OF
4-(N-ETHYL-N-2-HYDROXYETHYL)-2-METHYLPHENYLENE DIAMINE SULFATE (CD-4)

PURPOSE: The purpose of this study was to characterize the effects of CD-4 on selected target organs after two weeks of exposure.

TESTING FACILITY:
TOXICOLOGICAL SCIENCES SECTION
HEALTH AND ENVIRONMENT LABORATORIES (HAEL)
B-320 KODAK PARK
EASTMAN KODAK COMPANY
ROCHESTER, NY 14650

TEST ARTICLE CHARACTERIZATION:
CHEMICAL NAME: 4-(N-ethyl-N-2-hydroxyethyl)-2-methylphenylene diamine sulfate
ALTERNATE NAME: CD-4
HAEL NO.: 86-0039
EK ACC. NO.: 904984
CAS NO.: 25646-77-9
SRID OR LOT NO.: Lot A 15
EXPERIMENT NO.: 860039G2

PURITY, STABILITY, AND CONCENTRATION ANALYSES: The purity of the test material was determined in a previous four-week study using high pressure liquid chromatography (HPLC). Since the test sample was of high purity and was stable when neat, no further analyses were conducted. The purity was 99.1% before initiation and 99.0% after completion of that study. Stability of a solution of 2% CD-4 in distilled water was compared to that of a solution of 2% CD-4 in deoxygenated, distilled water. Distilled water was deoxygenated by bubbling nitrogen through it for 30 minutes. Samples from both solutions were analyzed by HPLC over a two-hour period. Five analyses of each sample were between 1.9 and 2.0% CD-4. Since both preparations were stable, with no difference observed between the vehicles, distilled water, which was not deoxygenated, was used as the vehicle for the test solutions. Test solutions were prepared daily and administered within two hours after preparation. Dose verification was performed by HPLC analyses on freshly prepared test solutions once each week (Days 0 and 7). Each solution was analyzed twice. The mean concentration of the four analyses was 2.1%.

TEST PROCEDURE: This study was conducted by methods comparable to OECD Guidelines for Testing of Chemicals TG-407, Repeated Dose Oral Toxicity - Rodent: 28-Day or 14-Day Study. Clinical pathology was not evaluated and only kidneys and heart were collected at necropsy.

TEST SUBSTANCE EXPOSURE: Rats were given 20, 40 or 60 mg/kg of the test material in distilled water for 11 doses over 15 days. Doses were given five days per week. The dosing concentrations were kept constant while the dosing volume was varied to achieve the various targeted doses. Controls received a dose of distilled water equal in volume to that of the highest test group.

ANIMALS: Five male and five female rats (CD®(SD)BR) from Charles River Laboratories, Wilmington, MA, were randomly assigned to each test group. Animals were isolated for approximately one week prior to testing. At the start of the study, rats were 5-6 (males) and 6 (females) weeks old and weighed (mean ± SD) 151 ± 3g (males) and 139 ± 4g (females). Rats were chosen for this study because they are a common representative species for toxicity studies.

HOUSING: Rats were housed in groups of five segregated by sex. The study was conducted in the vivarium area of Building 32. The study room was maintained at 70-74 °F and 60-63% relative humidity. A photoperiod of 12 hours from 6 a.m. to 6 p.m. was maintained. No other study was housed in the same room as this study. Cages and racks were washed once a week. Absorbent paper was changed under the cages at least three times a week.

FEED AND WATER: Agway Prolab Animal Diet (RMH 3200), certified ground chow was available ad libitum. Feed containers were cleaned weekly. Feed containers were refilled twice a week. Water was available ad libitum through an automatic watering system. The source of the water was the Monroe County Water Authority. No known contaminants in feed or water were expected which would interfere with the outcome of this study.

IDENTIFICATION: All rats were identified by a uniquely numbered metal ear tag.

RANDOMIZATION: All culling and randomization were done by computer-generated lists using the Automated Animal Toxicology System.

BODY AND FEED WEIGHT DETERMINATIONS: Body weights were collected on Days 0, 4, 7, and 14. Feed weights were collected on Days 2, 3, 4, 7, 11, and 14.

CLINICAL OBSERVATIONS: Every workday morning each rat was removed from its cage and examined by a trained technician. Every workday, post dose and afternoon, cageside observations were conducted. Cageside observation included, but was not limited to, examination of the hair, skin, eyes, motor activity, feces, and urine. Animals were checked for mortality on weekends.

NECROPSY: Rats dying during the course of the study were promptly necropsied. Rats surviving to the end of the study were fasted overnight, anesthetized with CO₂, and exsanguinated by severing the posterior vena cava. The kidneys and hearts were weighed and fixed in 10% buffered formalin. The kidneys were weighed together. Organ/body weight ratios were calculated. Significant necropsy lesions in other organs were also fixed in formalin. The kidneys, heart, and other organs with significant necropsy lesions were embedded in paraffin and processed for histopathology examination.

STATISTICAL PROCEDURES: Mean values were calculated for body weight, feed consumption, and organ weights. Mean data, except feed consumption and data from groups of only two rats, were evaluated statistically. For comparisons among three or more groups, the following computer-generated statistical tests were used: 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 was used in those cases where Bartlett's test indicated a lack of homogeneity of variances or for comparisons between two groups of data. Feed consumption was not analyzed statistically because the animals were group housed.

DATA STORAGE: The final report, tissues, paraffin blocks, slides, data sheets, and all non-perishable raw data were stored in the HAEL archives.

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 Environmental Protection Agency Good Laboratory Practice Standards 40 CFR Part 792, November 29, 1983, and OECD Principles of Good Laboratory Practice.

PROJECT PARTICIPANTS:

Study Director	G. V. Katz, Ph.D.
Study Technician	R. A. Moulton
Pathologist	M. S. Vlaovic, D.V.M., Ph.D.
Laboratory Animal Medicine	G. J. Hankinson, D.V.M., M.S.
Analytical Chemist	J. A. Zweigenbaum, M.S.
Report Author	G. V. Katz, Ph.D.

DATE OF STUDY INITIATION: July 13, 1987

PROTOCOL AND SOP DEVIATIONS: Three control female rats (Rats 171, 172, and 173) were dosed with 20 mg/kg CD-4 on the day before scheduled necropsy. Except for reducing the number of control animals available for evaluation, this deviation had no effect on the outcome of this study.

RESULTS

MORTALITY: Four males and four females exposed to 60 mg/kg died on Day 2 prior to receiving the third dose. The fifth male and female were moribund and, therefore, were euthanatized on Day 2 prior to the administration of the third dose. Four males exposed to 40 mg/kg died on Day 2 prior to the administration of the third dose. The fifth male in the group also died on Day 2 shortly after receiving the third dose. One moribund female, exposed to 40 mg/kg, was euthanatized on Day 2 shortly after administration of the third dose. Two other females in this dose group died on Day 3 prior to receiving the fourth dose. Two females exposed to 40 mg/kg survived to scheduled necropsy. All males and females receiving 20 mg/kg survived to the end of the study.

CLINICAL SIGNS: Red and/or brown discoloration of urine was observed on the dropping paper beneath the cages of males and females in all dose groups on Day 1. Discoloration also appeared beneath the cages of all rats exposed to 20 mg/kg on Days 8 and 9 and beneath the cage containing the two surviving females dosed with 40 mg/kg on Days 3, 7, and 8. The intensity of the discoloration was always greatest in the higher dosed animals.

Other clinical signs of toxicity in moribund males included lethargy, tremor, and hypothermia in one rat dosed with 40 mg/kg, and lethargy and hypothermia in two rats dosed with 60 mg/kg. Alopecia, seen on the skin of the backs in the latter two males, was probably not compound-related.

Other clinical signs of toxicity in moribund females included lethargy and tremor in one rat, and excessive weight loss in two others exposed to 40 mg/kg, and lethargy and hypothermia in one rat dosed with 60 mg/kg. Corneal opacity in the right eye of one rat dosed with 60 mg/kg was probably not compound-related.

Brown discoloration was seen on the dropping paper beneath the cage of three female control animals on Day 14 after dosing them with 20 mg/kg CD-4.

BODY WEIGHT AND FEED CONSUMPTION: Mean body weight was significantly ($P = 0.008$) decreased, by about 11-14%, in males dosed with 20 mg/kg. This was partially due to a decrease in the rate of weight gain for all 20 mg/kg males and especially Rat 160 which initially lost weight.

Mean body weight of females exposed to 20 mg/kg was slightly lower, but not statistically different from that of control values. However, three females did initially lose weight (Day 4), but their subsequent body weights were comparable to that of control values. Individual body weights of the two surviving females dosed with 40 mg/kg were significantly decreased during the first week, but were comparable to control values by the end of the study.

Feed consumption in the 20 mg/kg males and females was decreased during the first 11 days of the study, but was similar to control values by Day 14. Feed consumption in the two surviving females dosed with 40 mg/kg was depressed more than that of the 20 mg/kg group during the first week. Feed consumption for the 40 mg/kg group during the second week was comparable to the controls. Feed consumption for the control groups (27.3 and 27.6 mg/kg, for males and females, respectively) was unexpectedly high on Day 11.

TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS: Mean terminal body weights of males and females dosed with 20 mg/kg were slightly (about 10%) lower relative to their respective control values. The differences were statistically significant only for the males. The mean terminal body weight of the two surviving females dosed with 40 mg/kg was also lower (13%) than that of control values. The three control females dosed with 20 mg/kg CD-4 were included in the weight analysis; however, their terminal body weights did not appear to be affected by the exposure.

Mean absolute and relative kidney weights were increased (absolute, 30-50%; relative, 50-65%) in males and females exposed to 20 mg/kg. This was largely due to one male (#160) and one female (#176) whose weights were about twice that of the control animals. The mean absolute and relative kidney weights of the two surviving 40 mg/kg females were increased to an even greater extent, i.e., absolute, 134% and relative, 167%. None of the kidney weight differences were statistically significant at 20 or 40 mg/kg for either sex, but interpretation of data for the 20 mg/kg female group was confounded by dosing of three rats with CD-4.

Mean absolute heart weight of males dosed with 20 mg/kg was 15% ($P < 0.05$) less than the control value while relative heart weight was only 5% less. In females dosed with 20 mg/kg, the absolute heart weight was 2% less in comparison to control values, while the relative weight was 5% higher. Absolute and relative heart weights of the two surviving females dosed with 40 mg/kg were 4 and 19% greater than controls, respectively. None of the female heart weight differences were statistically significant. No consistent picture of heart weight gain or loss was seen in males or females.

GROSS PATHOLOGY: Compound-related necropsy changes were observed in the kidneys of all animals dosed with 40 or 60 mg/kg. These changes included kidneys with pale cortices and red medullae. In addition, these animals also exhibited hydrothorax, hydroperitoneum, and abdominal adipose tissue edema. Enlarged kidneys were seen in the two surviving females dosed with 40 mg/kg and in both sexes dosed with 20 mg/kg. Kidney changes were seen in each of the three female control rats which were given a single dose with 20 mg/kg test article on the day before scheduled necropsy; one showed enlarged kidneys with pale cortices and red medullae, a second showed pale renal cortices and medullae, and the third showed pale renal cortices.

HISTOPATHOLOGY: Compound-related histopathology was seen in the hearts and kidneys of animals dosed with 40 or 60 mg/kg which died or were killed in extremis. The heart lesions consisted of focal necrosis and focal inflammation of the right or left ventricle or the ventricular septum. Kidney lesions in these animals were primarily degenerative and included congestion, glomerular casts, tubular casts, tubular mineralization, and tubular dilatation. The most extensive lesions were observed in the proximal tubular epithelium and were characterized by diffuse necrosis, cytoplasmic vacuolation, and atrophy of the tubular epithelium.

In rats which survived the 15-day observation period, the location of lesions was in the proximal tubules, however, the type of lesion changed from degenerative to a predominantly regenerative type. Affected areas showed flat epithelium with basophilic hyperchromatic nuclei and dilated tubules. Proteinaceous and epithelial casts were present in most of the affected nephrons from proximal convoluted tubules to collecting ducts. A few of the proximal tubules, mostly within the inner cortex, exhibited necrosis affecting either single or most of the cells within the same cross section. A few of the proximal tubular cells showed cytoplasmic vacuoles. The interstitium showed edema, focal hemorrhage and focal accumulation of inflammatory cells. Most of the inflammatory cells were either lymphocytes or macrophages.

The three female control rats which were given a single dose of 20 mg/kg CD-4 showed congestion of renal vasculature, and diffuse necrosis and cytoplasmic vacuolation in the proximal convoluted tubules. Tubular casts were present in the proximal or proximal and distal convoluted tubules. Tubular dilatation affected proximal and distal convoluted tubules. In addition, edema and hemorrhage of the glandular gastric mucosa were also observed.

DISCUSSION AND INTERPRETATION

Rats, which either died or were killed within 72 hours after administration of 60 or 40 mg/kg/day CD-4, exhibited extensive necrosis of the proximal convoluted tubules. Similar lesions were found in three control animals within 24-hours of receiving a single dose of 20 mg/kg CD-4. The site of the lesion in surviving animals was still the proximal tubules, however, the type of lesion was characterized as predominantly regenerative. A no-effect-level for kidney lesions was not determined.

Cardiac lesions consisting of focal necrosis and infiltration by inflammatory cells were seen in a few animals of both sexes dosed with 60 or 40 mg/kg/day which died or were killed in extremis. Similar lesions have been described in the literature secondary to renal failure and uremia. Cardiovascular damage, for example, has been shown in studies of morbidity and mortality from cardiovascular disease in uremic patients¹ and in experimental studies of acute and chronic renal failure in the nephrectomized rat². The sequential development of cardiovascular pathology has been reported in the latter study. Cardiovascular lesions, which arose from acute and chronic renal failure (induced chemically or by nephrectomy), included focal myocardial edema and pericardial fibrin deposits and eventually myocardial necrosis and calcification. In the present study, cardiac lesions were not seen in any surviving animals in the groups dosed with 40 or 20 mg/kg CD-4.

The primary target-organ in the present study was the kidney, with one dose of 20 mg/kg CD-4 eliciting renal damage. Since a single 20 mg/kg dose was capable of producing renal damage within a 24-hour period, the toxicity resulting from repeated doses of 20 mg/kg CD-4 was probably due to repeated acute insults to the kidney rather than simple accumulation of CD-4 causing subacute toxicity. At high doses, cardiac lesions may occur as a secondary response to renal failure and uremia. A no-effect-level for kidney toxicity was not determined in the present study. The no-effect-level for cardiac lesions in the present study was 20 mg/kg/day.