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8(e) Coordinator
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Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

Dear Sir or Madam:

On October 21, 1987, Eastman Kodak Company submitted a copy of a 4-week oral sub-acute study on N,N-Diethyl-2-methyl-p-phenylenediamine monohydrochloride (CD-2), Kodak Document # TX-86-301. This report was sent as a follow-up to an 8(e) submission on 2-((4-amino-3-methylphenyl)ethylamino)-ethanol sulfate (EPA Document Control Number 8EHQ-1185-0575). Based on a telephone request by Mr. David R. Williams of the Chemical Screening Branch/ECAD, we are sending a second copy of this report since the first one has apparently been misplaced. If you have questions concerning this report, please contact me at the address below.

Sincerely,

Bruce D. Klaassen for RHB

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

RHB:JAF
Enc.

2309948
TX-86-301

FOUR WEEK ORAL TOXICITY STUDY OF CD-2 IN THE RAT
HAEL No. 86-0079 ACC. NO. 903478

BY L. G. BERNARD, A.A.S.

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

DATE OF STUDY COMPLETION SEPTEMPER 9, 1987

FOUR-WEEK ORAL TOXICITY STUDY OF CD-2 IN THE RAT

PURPOSE: To evaluate the subacute effects of CD-2 when given to rats orally for four weeks.

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: CD-2
ALTERNATE NAME: 4-N,N-diethyl-2-methylphenylenediamine monohydrochloride
HAEL NO.: 86-0079
EK ACC. NO.: 903478
CAS NO.: 2051-79-8
SRID OR LOT NO.: LOT C15
EXPERIMENT NO.: 860079G2

PURITY, STABILITY, and CONCENTRATION ANALYSES: The test compound was found to be 100% pure by high pressure liquid chromatography both before and after the study. A stability study conducted on a solution of 1.0% chemical in distilled water determined the solution stable for up to 26 hours, the longest time period studied. Test solutions for this study were 4.0% CD-2 in distilled water and were prepared daily. One solution was analyzed for concentration each week. The mean ± SD for these analysis was 4.45 ± 0.264%.

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, and Annex V B.7.

TEST SUBSTANCE EXPOSURE: Rats were given 20, 60, 120, or 200 mg/kg of the test material in distilled water 21 times over 29 days. Doses were given 5 days per week including holidays. 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 5 days prior to testing. At the start of the study, rats were approximately 5.5 (males) and 6.5 (females) weeks old and weighed (mean ± SD) approximately 169 ± 7 g (males) and 142 ± 4 g (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 320. The study room was maintained at 67-74 °F and 51-57% 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 under the cages was changed daily.

FEED AND WATER: Agway Prolab Animal Diet (RMH 3200), certified ground chow was fed *ad libitum*. Feed containers were cleaned weekly. Feed containers were refilled at least once a week. Water was supplied *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 unique metal ear tag.

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

BODY AND FEED WEIGHT DETERMINATIONS: Body weights were collected on Days 0, 3, 8, 14, 21, and 28. Feed weights were collected on Days 1, 3, 4, 8, 11, 14, 18, 21, 25, and 28.

CLINICAL OBSERVATIONS: Each rat was removed from its cage on the mornings of body weight measurement and examined by a trained technician. Every workday immediately after dosing and again in the afternoon cageside observations were conducted. Cageside observation included, but was not limited to, examination of the hair, skin, eyes, motor activity, feces, and urine.

HEMATOLOGY AND CLINICAL CHEMISTRY EXAMINATIONS: At the time of necropsy, blood was collected from the posterior vena cava while the rats were under CO_2 anesthesia. All assays were conducted by the Animal Clinical Analysis Group, HAEL. Hematology tests included: hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, differential white blood cell count, platelet count, red blood cell indices, and examination of the blood smears for cellular morphology. Clinical chemistry tests included: aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, total bilirubin, total protein, albumin, alkaline phosphatase, creatinine, urea nitrogen, and glucose.

URINALYSIS: One day prior to scheduled necropsy, all rats were placed in plastic (Nalgene®) metabolism cages for collection of 24 hr. urinary output. Water but not feed was available during the collection. Total collected urine was submitted to the Animal Clinical Analysis Group, HAEL for routine urinalysis and quantitation of N-acetyl glucosaminidase (NAG) activity.

NECROPSY: Rats were fasted overnight, anesthetized with CO_2, and exsanguinated by severing the posterior vena cava after collecting blood for analysis. Necropsies were conducted according to pathology SOP No. TP 180. The liver, kidneys, adrenal glands, testes, spleen, and thymus were weighed. Paired organs were weighed together. Organ/body weight ratios were calculated. The following organs were fixed in 10% buffered formalin: 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, testes, epididymides, male accessory sex glands, ovaries, vagina, uterus, Fallopian tubes, and gross lesions. All tissues were examined microscopically from the control and high-dose groups and target organs and gross lesions were examined from other groups.

STATISTICAL PROCEDURES: Mean values were calculated for body weight, feed consumption, organ weights, hematology, and clinical chemistries. All mean data, except feed consumption, 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 ($P \leq 0.05$) to indicate statistical significance. When the variances were not considered equal by the Bartlett's test ($P \leq 0.01$) the means of the test groups were compared individually to the control using a two-tailed t-test ($P \leq 0.05$). 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 Standard 40 CFR Part 792, November 29, 1983. This study was also conducted in accordance with OECD Principles of Good Laboratory Practice.

PROJECT PARTICIPANTS:

Study Director	G. J. Hankinson, D.V.M., M.S.
Study Technicia (s)	L. G. Bernard, A.A.S.
Necropsy Pathologist	M. S. Vlaovic, D.V.M., Ph.D.
Histopathologist	M. S. Vlaovic, D.V.M., Ph.D.
Laboratory Animal Medicine	G. J. Hankinson, D.V.M., M.S.
Hematologist/Clinical Chemist	R. E. Emmons, B.S.
Analytical Chemist	R. A. Schumacher, M.S.
Report Author	L. G. Bernard, A.A.S.

DATE OF STUDY INITIATION: August 25, 1986

PROTOCOL AND SOP DEVIATIONS: Test animals were weighed on Day 3 instead of Day 4. This change had no effect on the outcome of this study. A t-test was used in the evaluation of statistical significance when the variances were not considered equal by the Bartlett's test; this change was made to improve statistical analysis of the data.

RESULTS

MORTALITY: In the males at 200 mg/kg, one animal (R-565) died spontaneously on Day 4 and a second moribund animal (R-563) was euthanatized on Day 18. The other three males in this group survived to study termination on Day 29. No mortality was observed in the males at the other dose levels.

In the females at 200 mg/kg, four animals died spontaneously, two (R-588, R-589) on Day 1 and two (R-586, R-590) on Day 4. At 120 mg/kg, two animals (R-582, R-585) died spontaneously on Day 18. The remaining 200 mg/kg female and the three 120 mg/kg females survived until study termination. No mortality was observed in the females at the other three dose levels.

CLINICAL SIGNS: Urine stains under the cages of CD-2 treated rats were distinctly colored brown or blue. The brown stains may have been initially a pink-brown color. Brown stains were first seen within 24-hours after doses of 20 - 200 mg/kg except for the 20 mg/kg female group where the color did not appear until Day 10. Blue discoloration was evident during the first 24 hours at doses of 60 - 200 mg/kg , but not until 9 - 10 days after the first dose at 20 mg/kg. During urine collection for urinalysis, rats were housed singly and it was possible to determine that not all rats excreted colored urine. The frequency of brown or blue discoloration was not clearly dose-dependent although unequal group size and small numbers make direct comparisons tenuous. See the appendix for additional details.

CD-2 solutions prepared for this study were a light magenta but darkened to a much deeper magenta if left in a container overnight. When a solution of CD-2 was dropped onto clean cage paper, it dried to a brown color overnight. Both the increase in the intensity of the magenta (increased blue/purple) and the brown discoloration of the CD-2 when dried on the paper were probably due to autoxidation of CD-2. It is also probable that urinary color changes were due to the presence of oxidized CD-2. Whether this oxidation occurred in vivo or in vitro was not determined.

Excessive salivation was observed in all CD-2 dosed rats except for the 20 mg/kg females. Salivation was probably due to the taste of the chemical. Fecal material was decreased in quantity at 120 and 200 mg/kg in both sexes and at 60 mg/kg in females, and decreased in size at 200 mg/kg in males and females and at 120 mg/kg in females. Porphyrin nasal discharge was seen in the 200, 120, and 60 mg/kg females and in the 200, 120, and 20 mg/kg males. Porphyrin tears were observed in the 200 mg/kg males and the 200 and 120 mg/kg females. Alopecia was observed in the males at 200, 120, and 60 mg/kg and in the females at 200, 120, and 20 mg/kg.

Additional abnormalities observed, in the higher two dose levels only, included brown discoloration of back hair and/or skin by brown urine, urine soaked inguinal hair, weakness, dehydration, excessive weight loss, and poor body condition at 200 and 120 mg/kg in both sexes; swollen faces, pallor, and respiratory difficulty in males at 200 mg/kg and females at 120 and 200 mg/kg; general activity depression in 200 mg/kg males; hypothermia in 120 and 200 mg/kg females; abdominal distention in the 200 mg/kg females; and corneal opacity and tremors in the 120 mg/kg females.

Malocclusion, porphyrin nasal discharge, and porphyrin tears were observed in one control male.

BODY WEIGHT: Initial body weight declines were observed in the males at 120 and 200 mg/kg. This effect was slight at 120 mg/kg and moderate at 200 mg/kg. Though the absolute body weights remained lower than those of the controls, approximately 13-15%, the rate of body weight gain became comparable to that of the controls and the difference was not statistically significant at the end of the study. The females at 120 and 200 mg/kg showed initial moderate body weight declines. By Day 14, the surviving females had recovered and were of a weight comparable to that of the controls. Body weight gain in both the males and females at 20 and 60 mg/kg was comparable to that of the controls.

FEED CONSUMPTION: Initial feed consumption was moderately decreased at 60 mg/kg in females and at 120 mg/kg in both sexes, and greatly decreased at 200 mg/kg in both sexes. Feed consumption gradually returned to normal levels. Feed consumption was comparable to that of controls at 20 mg/kg in both sexes and at 60 mg/kg in males.

HEMATOLOGY: Abnormalities observed in hematologic parameters in the single male (R-563 200 mg/kg) euthanatized and bled on Day 18 consisted of: slight decreases in red blood cell count, hemoglobin concentration, and hematocrit, a great decrease in platelet count, and a great increase in prothrombin time. Alterations observed in white blood cell differential consisted of slight to moderate increases in neutrophil (polys), band, and metamyelocyte cell counts and a slight decrease in lymphocyte cell count. Anomalies noted in erythrocyte morphology consisted of increased polychromasia and the presence of Howell-Jolly bodies in RBCs.

Hemograms from rats which survived to the end of the study were considered normal although there were some minor statistical differences. The only difference among the male groups was a slight increase in relative myelocyte cell count at 200 and 60 mg/kg. Variability of myelocyte count within the groups was high and without any dose relationship.

For female groups, the only statistically significant differences were in: counts of platelets, percent polys, percent lymphocytes, and percent myelocytes. All other parameters were comparable among groups. The mean platelet count was statistically higher in the 120 mg/kg group when compared to the control. Poly and lymphocyte counts shifted in all treatment groups with higher poly and lower lymphocyte counts in all CD-2 dosed groups. When tested by Duncan's multiple range test, there was no difference in the magnitude of changes indicating a lack of a dose-response in the shift. Myelocyte counts were higher in the 120 mg/kg group, although the individual animal counts overlapped and actually amounted to the difference between counts of 1% vs. 2%; clinically these counts were the same. The one surviving female rat from the 200 mg/kg group had a hemogram similar to 120 mg/kg females and significantly different from the single moribund male rat.

CLINICAL CHEMISTRY: Serum clinical chemistry abnormalities observed in Rat 563 (200 mg/kg) consisted of slightly increased total bilirubin and creatinine levels, greatly increased aspartate aminotransferase, alanine aminotransferase, urea nitrogen, and sorbitol dehydrogenase levels, and moderately decreased glucose levels. Albumin, total protein, and alkaline phosphatase levels appeared normal.

In animals surviving to study termination, serum enzyme differences between control and treated groups were observed for sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). SDH levels for all treatment groups were slightly lower than control values although only SDH levels in males given 200 or 120 mg/kg were statistically different from the control.

AST levels in two male rats from the 200 and 120 mg/kg groups were increased but means for these groups were not statistically different from the control. The mean AST level in the male 60 mg/kg group was also slightly higher but not statistically different from the control. A single female at 60 mg/kg, three females at 120 mg/kg, and the surviving female at 200 mg/kg had increased levels of AST but none of the mean values were statistically different from the control because of small group size at the higher doses and variability in the response.

Alanine aminotransferase levels were slightly increased in both males and females at 200 mg/kg. One male at 120 mg/kg, two males at 200 mg/kg, and single females at 60, 120, and 200 mg/kg had increased ALT levels. Only the males at 200 mg/kg were statistically different from the control.

Other clinical chemistries including alkaline phosphatase, urea nitrogen, glucose, creatinine, total bilirubin, albumin, and total protein were comparable among all groups. The 20 mg/kg males and females had no differences which were statistically significantly different from the controls.

URINALYSIS: Specific gravity of the urine collected from the 60 mg/kg females was statistically higher than controls. Specific gravity of urine from the other dose levels of females and all treated males were comparable to the controls.

The N-acetyl glucosaminidase (NAG) activity and volumes of output were comparable to controls for both sexes at all treatment levels.

The appearance of the urine from the treated animals was similar to that from controls. Discolored urine was observed in one to three animals from each treatment group. The colors ranged from dark yellow to brown.

When the urine was examined using a dipstick method, Ames Multistix®-C (Miles Laboratories, Inc., Elkhart, IN), the levels of glucose, bilirubin, ketone, blood, protein, and urobilinogen were comparable to controls for both sexes at all dose levels. Increased levels of ascorbic acid activity were observed in the 20, 60, and 120 mg/kg females and in all treatment levels of males but excretion of the test article in the urine may have interfered with the results of this assay. Statistically significantly lower pH levels were present at 60, 120, and 200 mg/kg in males.

On microscopic examination of urinary sediment, the single 200 mg/kg female that survived to study termination had more white blood cells and epithelial cells present in the urine when compared to controls. No other microscopic abnormalities were observed at any dose level for either sex.

ORGAN WEIGHTS: The terminal body weights (TBW) of the 120 and 200 mg/kg males were slightly decreased. The TBW of the 20 and 60 mg/kg males and all treated females were comparable to controls.

Although absolute and relative (% body weight) liver weights were higher in all treatment groups compared to the controls, many of the differences were not statistically significant. Absolute liver weights for male groups did not follow a dose-response pattern, although at 200 mg/kg, the weight difference was probably compound-related. Relative male liver weights did follow a dose-response pattern and were statistically significant at all doses. For female groups, both absolute and relative liver weights were greater than control values at all dose levels and weight differences followed a dose-response pattern. Absolute and relative liver weights for the 120 mg/kg group and relative liver weight for the 60 mg/kg group were statistically significantly different.

The mean absolute testes weight of the 200 mg/kg males was slightly reduced ($P \leq 0.05$) and although the relative testes weights were less than control, there was no statistically significant difference between the high-dose and the control. All other absolute and relative weights were comparable to controls for both sexes.

GROSS PATHOLOGY: Treatment-related changes observed grossly at 200 mg/kg in the male that died on Day 4 or in the male that was euthanatized on Day 18 consisted of small seminal vesicles, yellow testes, dark or pale quadriceps femoris muscle, adipose tissue atrophy, enlarged and yellow liver, pale spleen, heart, and kidney cortex, thymus hemorrhage, alopecia, and porphyrin nasal discharge. The three males at this dose level that survived to study termination had alopecia, and small, soft testes. One 120 mg/kg male had small seminal vesicles and testes, and another had alopecia. No treatment-related changes were observed grossly in the other three males at 120 mg/kg or in the 20 or 60 mg/kg males.

Changes observed in the 200 mg/kg females that died on Day 1 consisted of dark quadriceps femoris muscle and pale spleen. Changes in those that died on Day 4 included dark quadriceps femoris muscle; atrophy of the bone marrow, mesenteric lymph nodes, and thymus; pale and small spleens; an enlarged liver; porphyrin tears; and a nasal discharge. The single 200 mg/kg female that survived until study termination had alopecia. Changes seen in at least one of the two 120 mg/kg females that died on Day 18 included white discoloration of the heart, atrophy of mesenteric lymph nodes, enlarged livers and adrenal glands, small and pale spleen, porphyrin nasal discharge, alopecia, and hydrothorax. The three survivors at this dose level had alopecia. No gross abnormalities were observed in the 20 and 60 mg/kg females.

HISTOPATHOLOGY: Abnormalities observed histologically in the 200 mg/kg male that died on Day 4 (R-565) included degeneration and diffuse chronic inflammation of the quadriceps femoris muscle and atrophy of the adipose tissue, thymic cortex, seminal vesicles, and prostate. Testicular lesions consisted of decreased numbers of spermatozoa and spermatids and atrophy of the seminiferous tubules. Spermatazoa in the epididymides were also decreased in numbers and appeared degenerated.

Treatment-related changes observed in Rat 563 (200 mg/kg male) were chronic focal inflammation of the heart, diffuse necrosis of the liver, cytoplasmic vacuolation and degeneration of the zona fasciculata of the adrenals, and atrophy of the splenic lymphatic follicles. All other organs in these two animals were comparable to the controls.

All three 200 mg/kg males that survived until study termination showed atrophy of the seminiferous tubules, and decreased numbers of spermatozoa, spermatids, and spermatocytes in the testes, and degeneration and decreased numbers (2/3) of spermatozoa in the epididymides. Single incidences of the following lesions were observed in these males: chronic focal inflammation of the heart, hyperkeratosis of the skin, hyperplasia of the interstitial cells of Leydig and spermatid giant cell formation in the testes, and the presence of a spermatic granuloma in the epididymides.

Treatment-related changes were observed in three of five males at 120 mg/kg. Chronic focal inflammation of the heart was observed in 2/5 animals. Testicular changes consisted of degeneration of spermatids (2/5), spermatozoa (1/5), and spermatocytes (1/5), decreased numbers of spermatids (1/5), spermatozoa (2/5), and spermatocytes (1/5), and spermatid giant cell formation (1/5). Spermatozoa were decreased in number and degenerated in the epididymides (2/5), and the seminal vesicles were atrophied (1/5). No compound-related abnormalities were observed histologically in the 60 or 20 mg/kg males.

Of the two 200 mg/kg females that died on Day 1, Rat 588 showed no treatment-related abnormalities and Rat 589 showed diffuse necrosis of the thymic cortex and atrophy of the splenic lymphatic follicles and red pulp.

Treatment-related changes observed in both 200 mg/kg females the died on Day 4 (R-586, R590) consisted of atrophy of the thymic cortex, mesenteric lymph nodes lymphatic follicles, and adipose tissue. Single occurences observed in these two females were diffuse necrosis of the thymic cortex, atrophy of the splenic lymphatic follicles and bone marrow, chronic focal inflammation of the heart, and hypertrophy of the liver.

Treatment-related changes observed in the single 200 mg/kg female to survive until study termination were restricted to chronic focal inflammation of the heart and quadriceps femoris muscle.

Hyperkeratosis of the skin was observed in both females at 120 mg/kg that died on Day 18. Additionally, Rat 585 had thymic hemorrhage and Rat 582 had chronic diffuse inflammation of the heart, diffuse necrosis of the liver, and atrophy of the mesenteric lymph node, and splenic lymphatic follicles.

The three 120 mg/kg females that survived until study termination showed chronic inflammation (3/3) of the heart, and hyperkeratosis (1/3) and acanthosis (1/3) of the skin. No treatment-related lesions were observed in females given the two lower doses, 60 or 20 mg/kg.

DISCUSSION AND INTERPRETATION

The test material when administered by gavage, in an aqueous solution, caused mortality at 200 mg/kg in both sexes (two males, four females) and at 120 mg/kg in two of five females. Deaths occurred after 2-5 doses of 200 mg/kg with one exception (15 doses) and after 14 or 15 doses of 120 mg/kg.

CD-2 exposed rats all excreted urine which was discolored brown or blue or dried to a brown color. The most likely cause of this color change in the urine was excretion of CD-2. Clinical manifestations of toxicity were generally non-specific resulting from discoloration of the haircoat, lack of grooming, and poor general body condition at the highest dose.

Feed consumption and consequently body weight gain were decreased in the two higher dose levels, 120 and 200 mg/kg, but rats which survived the initial toxicity of the test article gradually ate more feed and and gained weight at a rate comparable to controls or slightly greater than controls. Females given 60 mg/kg CD-2 ate less than controls but this did not result in a statistically significant difference in body weight. Males given 60 mg/kg CD-2 and both sexes given 20 mg/kg ate and gained body weight comparably to the control animals.

While hematologic abnormalities were noted in one male rat (200 mg/kg) euthanatized because it was moribund, these changes were probably agonal changes not indicative of specific red blood cell organ toxicity. All other hematologic changes were of a minor nature and not consistently related between dose levels or sexes.

Organ toxicity was present in the liver, heart, skeletal muscle, and testes. Hepatic toxicity was clearly apparent in Rats 563 (200 mg/kg male) and 582 (120 mg/kg female), both of which had diffuse necrosis of the liver. Serum enzymes for Rat 563 including AST, ALT, and SDH were markedly elevated. Rat 590 (200 mg/kg female) was the only other rat with compound-related microscopic alteration of the liver and this was limited to hepatocyte hypertrophy. Although the number of animals surviving the 200 mg/kg dose level was limited, absolute and relative liver weights were increased for males and females. At 120 mg/kg, livers were increased in weight for the females and probably the males since absolute liver weight was heavier than the control mean in spite of depressed body weight gain. Decreased body weight gain and heavier liver weights resulted in a statistically significant relative liver weight at 120 mg/kg. Absolute and relative liver weights were heavier than controls for the males and females at doses of 60 or 20 mg/kg but only the relative weights, except for the 20 mg/kg female group, were statistically significant. The absolute liver weights for the male 60 and 20 mg/kg groups were not dose-dependent.

Two serum enzymes which are frequently associated with liver toxicity, AST and ALT, were elevated in small numbers of rats from the 120 and 200 mg/kg dose levels and in one female from the 60 mg/kg dose level. All AST increases were accompanied by increased levels of ALT except for Rats 559 (120 mg/kg male)

and 562 (200 mg/kg male). Increases in AST levels in these two rats were relatively minor. Only ALT levels from the male 200 mg/kg group were statistically different from the control. Except for Rat 563, none of the AST or ALT elevations were accompanied by changes in SDH levels. In fact, SDH levels were generally lower than controls.

Interpretation of the AST and ALT levels is not straight forward in this study because tissue lesions were detected in the liver, heart, and skeletal muscles, all possible sources of enzyme activity. Except for Rat 563, AST and ALT levels were not associated with liver pathology; but, neither were they associated directly with pathology in the heart or the skeletal muscles. The most common association was between heart lesions and AST levels. Six of 10 rats with elevated AST had cardiac lesions, but rats with the highest AST levels did not have the most severe cardiac lesions. The most likely source of AST/ALT increases was the liver since the pattern of enzyme levels followed the pattern of increased liver weight and because significant ALT increases in the rat are more likely to be associated with the liver than the heart where ALT levels are relatively low. Since AST and ALT are both primarily in the cytoplasm in the liver and SDH is primarily mitochondrially bound, the pattern of enzyme elevation is consistent with hepatic cell membrane leakage rather than cell necrosis.

Heart lesions were seen at necropsy of two rats (one 200 mg/kg male and one 120 mg/kg female) on Day 18 of the study. The heart from the male was pale in color and the other heart was discolored white. Histological lesions were observed in the hearts of two males at 120 mg/kg, two males at 200 mg/kg, four females at 120 mg/kg, and two females at 200 mg/kg. Lower dose levels and the control did not have similar lesions, although, minimal inflammatory changes have occasionally been seen in control rats from other studies. Cardiac lesions were limited to single or multiple mononuclear cell inflammatory infiltrates primarily in the ventricular myocardium. Degenerative changes in cardiac muscle fibers were absent or minor.

Skeletal muscle lesions were seen at necropsy as muscles which were darker in color than normal, or in one instance, pale. Two males and four females given 200 mg/kg CD-2 showed this effect but no lower doses or controls did. Microscopic examination of two of the darkened muscle samples revealed chronic inflammation in both, accompanied by muscle fiber degeneration in one. Muscle which was not discolored at necropsy did not have similar microscopic lesions.

Lesions in the male reproductive system were relatively minor at 120 or 200 mg/kg and absent at lower levels. Only the 200 mg/kg male group had a statistically significant reduction in absolute testes weight although the relative weight was also less than the control. Three 120 mg/kg and four 200 mg/kg rats were affected. Affected testes generally had minimal to minor atrophy of the seminiferous epithelium with degenerative changes in developing spermatocytes, spermatids, and spermatozoa. Seminiferous atrophy was considered severe in one rat. Epididymal spermatozoa also showed degenerative changes in most rats with testicular changes. Accessory sex organs, primarily seminal vesicles, were atrophied in two rats.

In contrast to CD-4, renal lesions were absent in spite of the apparent excretion of CD-2 by the kidneys. Kidney weights, serum clinical chemistries, urinalysis including N-acetyl glucosaminidase analysis, and pathology examinations were all essentially normal with no indication of renal toxicity.

The most significant toxicity associated with CD-2 exposure was considered to involve the liver. The no-observed-effect-level was considered to be 20 mg/kg/day. At this dose level, both males and females ate and gained body weight at rates equal to or better than the controls. The only statistically significant difference from controls at this dose level was a slightly higher relative liver weight for the males. Since liver weights were not dose-dependent at the lower dose levels, these data were not considered sufficient to indicate either a toxic or an adaptive response to CD-2 exposure.

TABLES: Tables 1 and 2 summarize the results of this study. Tables, including means and individual data points for body weight, feed consumption, hematology, clinical chemistries, and organ weights, and reports of individual contributors are attached.

SIGNATURE PAGE

Lisa Seyb Bernard — 9-9-87
REPORT AUTHOR — DATE

Gordon J Hankinson — Sept. 9, 1987
STUDY DIRECTOR — DATE

John C Wyllie — Sept 9, 1987
SUPERVISOR OF TOXICOLOGICAL SCIENCES SECTION — DATE

[illegible] — 9/4/87
QUALITY ASSURANCE UNIT — DATE