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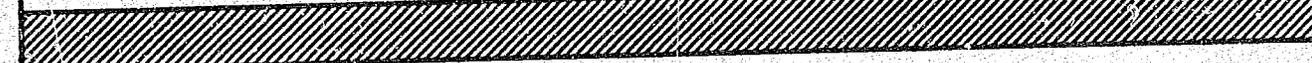
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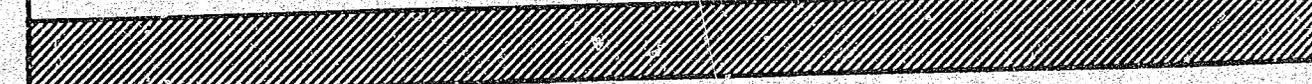
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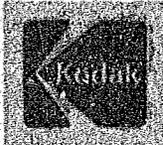
Contractor



Document Title
**THE DISPOSITION AND KINETICS OF CD-4 SULFATE COLOR DEVELOPER
IN SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE ORAL DOSE &
13-WEEK ORAL TOXICITY WITH COVER LETTERS DATED 121388 &
122888**



Chemical Category
CD-4 SULFATE



31705

8EHQ-1288-0575

POCN

88-860000-32
89-890000-56

EPA-OTS



0006000841

CONTAINS NO CBI

December 13, 1988

8(e) Coordinator
Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

63 DEC 19 PM 2:36
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Dear Sir or Madam:

The following test reports are being voluntarily provided as follow-up to a previous 8(e) submission for CD-4, also known as 4-(N-ethyl-N-2-hydroxyethyl)-2-methylphenylene diamine sulfate (EPA Document Control Number 8EHQ-1185-0575). The enclosed reports are entitled: "A Thirteen-Week Oral Toxicity Study of 4-(N-Ethyl-N-2-Hydroxyethyl)-2-Methylphenylene Diamine Sulfate (CD-4) in the Rat", and "The Disposition and Kinetics of CD-4 Sulfate Color Developer in Male and Female Sprague Dawley Rats Following a Single Oral Dose". The reports present results from a 13-week repeated dose oral toxicity study on CD-4 and on a disposition and kinetics study for the compound. We are forwarding copies of both studies for your records. If you have questions concerning these reports, please contact me at the address below.

Sincerely,

R. Hays Bell

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

RHB:WLH
Enc.



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December 28, 1988

8EHQ-1185-0575

ADDENDUM

PDN 88-860000032

89-890000063

8(e) Coordinator
Document Processing Center (TS-790)
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Environmental Protection Agency
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EPA-OTS



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Dear Sir or Madam:

This letter is in response to a telephone call from Dr. Judy Loranger of the Office of Toxic Substances in which she requested copies of pages that are missing from a test report provided on December 13, 1988. The test report reflects a study conducted as follow-up to a previous 8(e) submission for CD-4, also known as 4-(N-ethyl-N-2-hydroxyethyl)-2-methylphenylene diamine sulfate (EPA Document Control Number 8EHQ-1185-0575). The report is entitled: "A Thirteen-Week Oral Toxicity Study of 4-(N-Ethyl-N-2-Hydroxyethyl)-2-Methylphenylene Diamine Sulfate (CD-4) in the Rat". Enclosed please find pages 126 to 163 from the 13-week study. If you have questions concerning this letter, please contact me at the address below.

Sincerely,

R. Hays Bell

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

cc: Dr. J. Loranger

RHB:WLH
Enc.



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244961V
TX-88-159

FINAL REPORT

THE DISPOSITION AND KINETICS OF CD-4 SULFATE COLOR DEVELOPER
IN MALE AND FEMALE SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE ORAL DOSE

Accession No.: 904984

HAEL No.: 86-0039

David A. Morgott Ph.D., Mary Lou Lyon A.A.S. and Derek Guest Ph.D.

BIOCHEMICAL TOXICOLOGY SECTION
TOXICOLOGICAL SCIENCES LABORATORY
HEALTH AND ENVIRONMENT LABORATORIES
EASTMAN KODAK COMPANY
KODAK PARK (Bldg. 320)
1100 RIDGEWAY AVENUE
ROCHESTER, NEW YORK 14652-3615

DECEMBER 2, 1988

PREFACE

This report presents data compiled during an investigation of the test compound specified. All raw data, standard operating procedures, protocols and other documents pertaining to this investigation are maintained in archival storage in the Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY. Unless noted otherwise, all aspects of this study were performed in accordance with Good Laboratory Practice (GLP) Regulations of the U.S. Environmental Protection Agency (40 CFR Part 792). There were no known deviations from the GLP Regulations that significantly affected the quality of this study. Health and safety procedures established by the Toxicological Sciences Laboratory for handling potentially toxic and radioactive chemicals were observed throughout these studies. Gloves and protective clothing were worn when working with dose solutions, excreta, and biological specimens that contained the test compound.

**THE DISPOSITION AND KINETICS OF CD-4 SULFATE COLOR DEVELOPER
IN MALE AND FEMALE SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE ORAL DOSE**

Accession No.: 904984

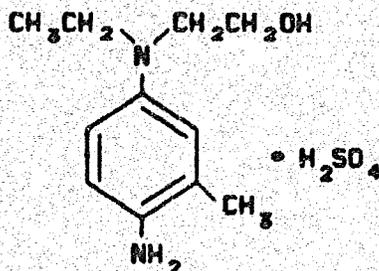
HAEL No.: 86-0039

ABSTRACT

The disposition and pharmacokinetics of a single 2 mg/kg oral dose of radiolabelled [^{14}C]CD-4 sulfate was examined in Sprague-Dawley rats of each sex. Urine, feces, and expired volatiles were collected at 12 to 24 hour intervals for 3 days following treatment, and analyzed for total radioactivity. Mass balance calculations showed the urinary route of excretion to predominate in both male and female rats with the average recovery being 66.7 and 73.0% of the dose, respectively. Approximately 90-98% of this urinary radioactivity was present in the initial 12-hour specimen. Total fecal radioactivity averaged less than 20%, and expired volatiles less than 0.06% of the dose in each sex. Little or no residual radioactivity was detected in selected organs and tissues upon termination of the study at 72 hours. Thin layer chromatography and autoradiography of the 12-hour urine samples showed the presence of a major metabolite averaging 22-24% of the administered dose in each sex. An additional four metabolites were detected that averaged about 6-12% of the dose in each sex. Insufficient material was present in the urine to allow for identification of the major metabolite. Pharmacokinetic data indicated that male and female Sprague-Dawley rats absorb and eliminate radiolabelled CD-4 equivalents very rapidly and at similar rates following oral treatment. There were no distinct sex-related differences in any of the pharmacokinetic parameters examined. A two-compartment open model best described the kinetics of CD-4 in both male and female rats. The half-life for elimination of the radioactivity was approximately 18 minutes and 7 hours for the rapid and slow compartments, respectively. These half-life values were consistent with the rapid urinary excretion observed in the disposition study. The kinetic data did not provide an explanation for previously observed differences in the nephrotoxicity of CD-4 sulfate between male and female rats. However, the data did indicate that CD-4 sulfate is rapidly and extensively metabolized in the rat with only limited potential for day-to-day accumulation of the parent compound and its metabolites upon repeated exposures.

INTRODUCTION

CD-4 sulfate (4-[N-ethyl-N-2-hydroxyethyl]-2-methylphenylenediamine sulfate) is a substituted phenylenediamine having the following physical and chemical properties at room temperature (1):



appearance.....pink powder

formula..... $C_{11}H_{18}N_2O \cdot H_2SO_4$

melting point.....157°C

odor.....faint sweetish

mol. weight.....292.4

vapor pressure.....negligible

The commercial grade material is used as a photographic developing agent for processing color negative film. CD-4 sulfate is present as one of three ingredients in Kodak Flexicolor™ developer, and is packaged as an unmixed liquid concentrate containing a proprietary antioxidant. When oxidized by the exposed silver ions in film, the compound forms an iminoquinone intermediate which reacts with an organic coupler to yield a final colored image. In solution, the highly water soluble sulfate salt is susceptible to air oxidation, especially upon neutralization with base. The primary route of human exposure to CD-4 would be expected to be through direct skin contact with the developer solution.

Previous oral disposition studies suggest rapid urinary elimination of uniformly ring-labelled [^{14}C]CD-4 sulfate in rats (2). Groups of male Sprague-Dawley rats administered an oral dose of 55 to 65 mg/kg CD-4 sulfate (ca. 3 μCi /rat) and sacrificed at either 2, 4, 8, or 16 days post-treatment showed that:

- i) urinary radioactivity levels peaked at about 45 to 55% of the dose after 2 days;
- ii) fecal radioactivity remained relatively constant at approximately 15 to 20% of the dose;
- iii) less than 0.1% of the dose was excreted in the expired air as $^{14}\text{CO}_2$;
- iv) tissue radioactivity was highest in the liver, kidneys, and spleen of rats examined after 2 to 8 days.

Analysis of the urine specimens for CD-4 metabolites was hindered by the low levels of radioactivity and by the relatively insensitive analytical techniques employed. Consequently, only one major and one questionable minor metabolite were detected by thin-layer chromatography and radiochromatogram scanning. Because the total recovery of radioactivity never exceeded 75% of the administered dose, the results from this study provided only an initial estimate of the probable rates and routes of CD-4 elimination in the rat. In addition, the use of nephrotoxic dosages of CD-4 sulfate limited the usefulness of the data, and prevented any meaningful extrapolation to situations involving nontoxic exposures. The following investigation has sought to remedy many of these shortcomings, while at the same time expanding upon the scope to include an examination of CD-4 blood kinetics in rats of both sexes.

The purpose of the following study was to examine the disposition and kinetics of a single oral dose of [^{14}C]CD-4 sulfate in male and female Sprague-Dawley rats. The routes, rates and patterns of CD-4 elimination were examined following the administration of a subtoxic dose of the ^{14}C -labelled material. The oral route of administration was selected on the basis of recent dermal absorption studies showing little or no penetration of CD-4 sulfate through rat skin (3), and on the availability of rat toxicity data using this route of exposure (4,5).

MATERIALS AND METHODS

Animals Groups of male and female Sprague-Dawley rats [CrI:CD[®](SD)BR] were purchased from the Charles River Laboratories (Wilmington, MA) and examined by qualified technical staff upon arrival. Animals were held in isolation for at least five days in a controlled-access vivarium accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). During this time, the animals were given uniquely numbered ear tags and allowed tap water (Monroe County Water Authority) and a Certified Rodent Diet (Agway[®] Prolab[™], RMH 3000) *ad libitum*. Care, husbandry, and feeding of the animals conformed to the standard operating procedures of the Toxicological Sciences Laboratory. Test animals were examined by a licensed veterinarian before being assigned to a study group. The weight range of the test animals was between 170 and 250 g at the time of use. The species and strain of animals used in this study were selected to support previously conducted CD-4 sulfate toxicity studies.

Test Compound Uniformly ring-labelled [ring-U-¹⁴C]4-(N-ethyl-N-2-hydroxy-ethyl)2-methylphenylenediamine sulfate (CD-4 sulfate) was synthesized by Amersham Corp. (Arlington Heights, IL) at a stated specific radioactivity of 18.2 mCi/mmol and radiochemical purity of 98%. The radiochemical purity of [¹⁴C]CD-4 was checked by liquid scintillation spectrometry (LSS) of serial column fractions obtained following separation of the N-acetylated derivative by high performance liquid chromatography (HPLC). Separation was accomplished on a 25 cm polystyrene divinylbenzene copolymer reversed-phase column (PRP-1, Hamilton Co.) using gradient elution (1 ml/min) with methanol and 20 mM sodium acetate buffer (pH 4.0) that contained 5 mM heptane sulfonic acid as a counter ion. The stock preparation of [¹⁴C]CD-4 sulfate was found to be 92% radiochemically pure by this method.

Dosing Solutions Rats used in the disposition and pharmacokinetic portions of the study were each administered a nominal oral dose of 2 mg/kg

CD-4 sulfate in a vehicle volume of 2 ml/kg. Dosing solutions were prepared on the day of treatment using distilled-deionized water that was boiled, and then purged with nitrogen to remove dissolved oxygen. The dosing solution was not diluted with unlabelled CD-4 sulfate and no pH adjustments were made after dissolution of the test compound. The final dosing solution was analyzed in triplicate for both total radioactivity using LSS, and CD-4 concentration using HPLC with ultraviolet (UV) detection (3). On the basis of these measurements, the actual administered dosage of [14 C]CD-4 sulfate ranged from 1.6-2.1 mg/kg (20-30 μ Ci/rat).

Treatment Groups of five male and five female rats were used to examine CD-4 sulfate disposition, and groups of four rats of each sex to study the kinetics. Rats used in both the disposition and kinetic studies were placed in individual all glass metabolism cages (Metabowl[®] Mk III, Jencons Scientific Ltd.) for approximately 48 hours prior to treatment. During this acclimation period, urine and fecal control specimens were collected from some of the animals and food and water were made freely available. Airflow through the chambers was adjusted to about 500 ml/min. Feed was withdrawn for a period of about 6-8 hours before and after treatment. The test compound was administered by gavage with the actual dosage volume calculated from the initial and final weight of the dosing syringe. The treatment time for each rat was staggered by 5-10 minutes to facilitate subsequent specimen collection.

Sample Collection Mass balance determinations were based on the collection of urine, feces, expired volatiles, and tissue specimens. Excreta specimens were collected at intervals of 12, 24, 48, and 72 hours post-treatment. Samples of CO₂ were not collected, since previous studies have shown that ring-labelled CD-4 is not metabolized via this pathway (2). All tissue and excreta specimens were weighed into tared containers and stored frozen until analyzed by LSS. A pair of silica gel traps, used to adsorb any volatile metabolites in the expired air, was charged with approximately 15-20 g of fresh silica at each collection interval. The silica from each trap was mixed with about 20 ml of

methanol to desorb any volatile metabolites. Urine specimens were collected over ice in light protected vessels containing 5-10 mg of ascorbic acid to prevent oxidation and decomposition of the excreted metabolites. After collection, the urine specimens were stabilized by adding sufficient 5.0 N hydrochloric acid to decrease the pH to approximately 1.0-2.0. Prior to sacrifice at 72 hours, animals were exsanguinated from the abdominal aorta while under carbon dioxide anesthesia. The following organs were removed for analysis of residual radioactivity: heart, liver, spleen, pancreas, thymus, and kidneys. Tissue specimens were stored frozen along with the carcass (ca. -70°C) for analysis at a later time.

The absorption and elimination of radiolabelled CD-4 equivalents from the blood were determined in a separate group of rats. Animals were housed, acclimated and treated in the same manner as those used for the metabolism portion of the study. Heparinized blood specimens of 100-200 µl were collected by retro-orbital bleeding at the following target times after treatment: 0.25, 0.50, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0, and 9.0 hours.

Sample Analysis Samples of excreta, tissues, and expired volatiles were analyzed in triplicate for total radioactivity by LSS using 10-20 ml of a water-miscible scintillation cocktail (Ready-To-Use II™, Eastman Kodak Co.). Urine, whole blood, thymus, and spleen were analyzed directly, while the feces and remaining organ specimens were oxidized as an aqueous homogenate before being counted. Quench correction was performed automatically by the external standards-ratio technique. An aliquot of urine from each collection was assayed for creatinine and N-acetylglucosaminidase activity to determine whether any treatment-related nephrotoxicity had occurred.

Metabolite analysis was performed on an aliquot of urine from each collection interval. Because of potential metabolite instability, urine specimens were analyzed directly without prior sample preparation. Selective hydrolysis of the urine specimens was accomplished with

β -glucuronidase (from bovine liver, 1651 units/mg Calbiochem Behring) and two types of arylsulfatase (type V from limpets, 11.6 units/mg, and type H-1 from helix pomatia, 21.3 units/mg, Sigma Chemical Co.).

Quantitative analysis of the urinary metabolites was performed by thin-layer chromatography (TLC) and autoradiography. Undiluted urine samples were spotted on TLC plates (type LK6DF, Whatman Inc.) and developed using chloroform/methanol/ammonium hydroxide (70:30:2). The developed TLC plates were dried and covered with plastic film (Handi Wrap[®] II, Dow Chemical Co.) to prevent contamination of the autoradiogram. A sheet of X-ray film (X-Omat[™] XAR-5, Eastman Kodak Co.) was placed in contact with the TLC plate and stored in a cassette for 2 to 18 days at approximately -70°C. The exposed film was developed in an automatic film processor to visualize the bands of radioactivity as discrete zones of darkened film. The number, position, and intensity of the zones on the film provided a measure of the [¹⁴C]CD-4 metabolite pattern on the original TLC plate.

The relative amounts of the individual metabolites were measured by densitometric analysis of the autoradiograms. Calibration of the densitometer was accomplished with autoradiograms prepared from TLC plates spotted with known amounts of radioactivity. Solutions of [ring-U-¹⁴C] 4-chloro-3-nitroaniline (95% radiochemical purity, 6.6 mCi/mole, Amersham Corp.) were prepared in acetone/water and applied to the plates (25 μ l/lane) in amounts ranging from about 250 to 650,000 dpm. After development in a mobile phase of toluene/ethanol/ammonium hydroxide (80:20:1), the plates were exposed to X-ray film for 2 to 12 days as described for the urine samples. A three dimensional plot of the integrated results from the densitometer versus both exposure duration and applied radioactivity showed a linear relationship between band density and the length of time the film was left in contact with the plate (Figure 1). The response was linear at each of the radioactivity levels examined. The relationship between band density and applied radioactivity showed deviations from linearity at levels of radioactivity above 60,000

dpm. The degree of departure from linearity became greater as the length of the exposure was increased. However, bands containing from about 250 to 50,000 dpm of radioactivity produced an apparent linear increase in film opacity for all of the exposure durations examined.

Data Analysis Metabolism data were summarized using descriptive statistics when necessary (e.g. mean, standard deviation, etc.). A full description of the mathematical manipulations used to analyze much of the metabolic data is presented in the Appendix. Kinetic data were fit to a two-compartment mathematical model using a weighted least squares regression algorithm (NONLIN84, Statistical Consultants, Inc.). Estimates for the area under the blood concentration-time curve, blood half-life, rate of clearance, and rate constants for absorption and elimination were determined after optimization of the model.

Instrumentation HPLC separations were performed using a Waters modular liquid chromatographic system composed of three model 510 solvent delivery pumps, a model 510E sample processor (WISP), a model 720 system controller, and a model 730 data module. A Kratos Spectroflow 773 absorbance detector and a LKB Bromma 2211 Superrac fraction collector were used in conjunction with the HPLC system. LSS determinations were accomplished on an LKB Wallac RackBeta 1217 liquid scintillation counter. A Packard 306 sample oxidizer was used to combust tissue and excreta specimens. Exposed autoradiographic films were processed in a Kodak RP X-Omat Film Processor model M6N. A Cliniscan™ densitometer was used to measure exposure density on the thin-layer autoradiographic films.

RESULTS

A 2 mg/kg dose of CD-4 sulfate caused no apparent renal damage to either the male or female rats used in this study. The levels of N-acetylglucosaminidase and creatinine in the urine specimens from treated animals showed no distinct time-related trends or sex-related differences. In

addition, the data compared favorably with historical control values. Historical averages (n=5) for urine N-acetylglucosaminidase activity in male and female Sprague-Dawley rats are 343.3 ± 121.9 and 209.5 ± 12.1 mUnits/24 hrs, respectively (4). These compare favorably with the overall averages of 256.4 ± 56.4 for the male and 235.8 ± 48.3 mUnits/24 hrs for the female test animals administered CD-4 sulfate. Individual results for all of the urine specimens are presented in the Appendix.

Mass balance calculations showed the urinary route of excretion to predominate in both male and female rats. Neglecting the cage wash data, the average 3-day recovery of urinary radioactivity represented 67 and 73% of the dose for male and female rats, respectively (Tables 1, 2). These recoveries increased by an average of 7% when the radioactivity in the cage washings was included in the urine recovery. Approximately 96% of the urine radioactivity was present in the initial 12-hour specimen. Total fecal radioactivity averaged 13-19% of the dose, with greater than 90% of this amount recovered within 24 hours of treatment. Expired volatiles accounted for less than 0.06% of the dose in each sex. In addition, little or no residual radioactivity was detected in the organs and tissues removed upon termination of the study at 72 hours. The total amount of radioactivity remaining in the animals at sacrifice (tissues and carcass) averaged 0.1-0.2% of the dose. The overall mean recovery was found to be 92.7 and 93.2% of the administered radioactivity for males and females, respectively. Collection and analysis results for individual specimens are presented in the Appendices.

Thin-layer chromatography and autoradiography of urine samples from male and female rats revealed at least eight separate bands of radioactivity (bands A-H), none of which co-chromatographed with the parent compound. The individual metabolite bands are presented in Tables 3 and 4 according to their relative position on the plate (i.e., metabolite A is the most polar and has the lowest R_f). The amount of radioactivity associated with each band was calculated as a percentage of the administered dose and then totaled for the four collection intervals examined (12, 24, 48, and

72 hours). An average of 22-24% of the dose was associated with a single band (metabolite C) in the 12-hour urine specimens from male and female rats. This band constituted the major urinary metabolite of CD-4 sulfate and was noticeably absent in urine specimens collected beyond 12 hours. Metabolite C appeared to have been converted to less polar metabolites in later urine specimen. Though precautions were taken to protect the metabolites against degradation, oxidative changes during sample storage or chromatography may be at least partially responsible for the shift in R_f value for metabolite C. Moderate amounts of several other metabolites were present in many of the urine specimens. Metabolites A, D, G, and H each averaged about 6-12% of the administered dose for both sexes. The remaining three minor metabolites (B, E, and F) each averaged less than 6% of the administered dose. Qualitative identification of the major urinary metabolites of CD-4 sulfate was hampered by their instability, and by the small amounts available for isolation and mass spectral analysis. Attempts at isolating metabolite C from rat urine using liquid (ethyl acetate) or solid (XAD-2) phase extractions led to its decomposition to a compound having the same R_f as metabolite H. Likewise, the collection and lyophilization of the eluent from a TLC or HPLC separation revealed substantial degradation of metabolite C after evaporation of the solvent. In contrast, overnight incubation of a buffered urine sample (37°C, pH 5.0) with either β -glucuronidase or sulfatase did not noticeably affect the TLC metabolite profile. The urine concentration of metabolite C was calculated to be less than 10 $\mu\text{g/ml}$ using an average urine volume of 5 ml for a 12-hour collection and a metabolite recovery value of 15% (0.70×0.22). This concentration approached the UV limit of detection and required that at least 10 ml of urine be used to isolate and purify sufficient amounts of metabolite C. Processing such a large volume of urine for a metabolite that was susceptible to decomposition proved to be infeasible.

Analysis of the pharmacokinetic data showed that male and female Sprague-Dawley rats absorbed and eliminated radiolabelled CD-4 equivalents very rapidly, and at similar rates following oral treatment. A

two-compartment open model best described the blood kinetics of CD-4 in both male and female rats. The rate constants, rate coefficients, and half-lives describing the pharmacokinetic behavior of CD-4 sulfate in rats have been summarized in Tables 5 and 6, while graphical and tabular presentations of the results from each individual animal have been placed in the Appendices. Peak blood levels tended to coincide with the collection of the first blood sample at 15 minutes post-treatment. Consequently, the shape of the blood absorption curve was not well defined. However, modeling of the available data suggested that the average half-life for gastrointestinal absorption ($k_{01} t_{1/2}$) was no greater than 12 minutes. The average half-life for elimination of CD-4 equivalents from the blood was found to be approximately 18 minutes and 7 hours for the rapid ($\alpha t_{1/2}$) and slow ($\beta t_{1/2}$) compartments, respectively. The volume of distribution (V_d) and the area under the blood CD-4 versus time curves (AUC) for male and female rats were not noticeably different. The V_d averaged between 800 and 950 g/kg, while the AUC was between 1.9 and 2.5 $\mu\text{g equiv.}\cdot\text{hr/g}$ for both sexes.

DISCUSSION

CD-4 sulfate has been found to be a potent nephrotoxic agent capable of causing renal tubular necrosis in rats receiving a single oral dose (5). The acute oral LD_{50} of CD-4 sulfate in fasted male and female Sprague-Dawley rats was in the range of 25 to 50 mg/kg. Rats that succumbed during the 14-day observation period were generally found to possess pale whitish kidneys that revealed proximal tubular necrosis in the inner renal cortex upon histopathologic analysis. Apparent secondary organ damage was also noted in the heart, spleen and pancreas. Many of the animals were found to excrete a reddish-brown urine. The no-observable-effect-level (NOEL) for the kidney lesion was 3-6 mg/kg for male and female rats. Results from a recent two-week range finding study conducted as part of a more extensive subacute 90-day study also showed renal damage in the treated animals (6). The administration of CD-4 sulfate to rats at

dosages of 0, 20, 40, and 60 mg/kg/day caused mortality, kidney weight gain, and tubular necrosis at each of these dosages.

In contrast, a recent aerosol inhalation study with male and female Sprague-Dawley rats did not result in any gross or microscopic evidence of nephrotoxicity or cardiotoxicity relative to control animals (7). The animals received a single 6-hour exposure to CD-4 particulates at a chamber concentration of 164 mg/m³. The respirable concentration of CD-4 was estimated to be 82 mg/m³ based on a respirable fraction of 50% (particle diameter less than 4.7 µm). Assuming 100% uptake of the respirable fraction, the author calculated the absorbed dose of CD-4 to be 12-15 mg/kg, which was within the range that caused renal damage by the oral route of administration.

A slight sex-difference was observed in a recent four-week subacute gavage study with CD-4 sulfate (8). Results from the study showed that unfasted male and female rats administered 0, 0.1, 1.0, and 10 mg/kg/day of CD-4 sulfate (5 days/week) had normal hematology, clinical chemistry, and urinalysis values at the lowest three dose levels. However, several of the female, but not male rats, receiving the 10 mg/kg/day dose had elevated white blood cell counts and N-acetylglucosaminidase activity in their urines. In contrast, the results from the current study show CD-4 elimination to be nearly the same for both male and female rats receiving a single oral dose. Since the toxicity study involved repetitive dosing, the slightly higher susceptibility of female rats may reflect a small differential change in sensitivity due to enzyme induction or receptor affinity. If so, multiple exposures would be expected to affect the pharmacokinetics of CD-4 differently in each sex.

The pharmacokinetic data suggests that the potential for day-to-day accumulation of CD-4 and its metabolites upon repeated oral treatment would be low for the rat. The clearance of CD-4 equivalents from the blood of male and female rats was best described by a two-compartment open model. The rate of clearance of CD-4 equivalents from the blood was in

agreement with data showing the rapid elimination of biotransformation products in the urine. Since parent CD-4 kinetics were not measured apart from total blood radioactivity, little can be said of the rate of CD-4 metabolism in relation to the time of onset of the renal lesion. Consequently, the role metabolism plays in affecting the renal damage to CD-4 sulfate was not apparent from the results of this study.

The presence of unstable metabolites in the urine of rats administered CD-4 sulfate may be associated with the production of electrophilic intermediates that are responsible for the production of the renal lesion. However, these labile metabolites may simply reflect the strong tendency of p-phenylenediamine-type color developers to oxidize when in solution. This latter possibility is supported by data showing that the profile of labelled metabolites in the urine of rats given any of a number of different p-phenylenediamine-type developers was altered by storage of the specimens in the freezer (2). These same studies showed a time-dependent qualitative change in the urinary metabolite pattern of rats administered the ring demethylated congener of CD-4. These animals were found to excrete a major urinary metabolite which was located at either of two positions on a TLC plate. A similar effect was observed in the present study with the appearance of metabolite D in place of metabolite C for urine specimens collected beyond 12 hours. Since this obvious change in the chemical structure of metabolite C may simply represent a sample handling artifact, the biological significance of the change is difficult to ascertain.

CD-2 hydrochloride is a close structural analog of CD-4 that does not cause serious renal toxicity in Sprague-Dawley rats. The LD₅₀ of CD-2 hydrochloride in male and female rats was found to be approximately 110-130 mg/kg (9). Clinical signs of toxicity included a pink discoloration of the urine, prostration, and convulsions. A four-week gavage study with CD-2 hydrochloride at dosages of 20, 60, 120, and 200 mg/kg/day caused a treatment-related decline in food consumption and evidence of liver damage at the highest two dose levels; but no