

Reviewed by: Timothy F. McMahon, Ph.D. *T.F. McMahon 5/5/92*
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *J.M.I. 5/5/92*
Section I, Toxicology Branch II (H7509C)

009629

Data Evaluation Report

Study type: Metabolism (85-1)

EPA identification numbers: EPA MRID numbers: 416978-01
Tox. Chem. number: 632
HED project numbers: 1-0935

Laboratory Project numbers: EHL 88083

Test material: 1,4-dichlorobenzene (unlabeled); [¹⁴C] 1,4-dichlorobenzene

Synonyms: para-dichlorobenzene; 1,4-DCB

Testing Facilities: Monsanto Company Environmental Health Laboratory
St. Louis, Missouri 63110

Sponsor: Midwest Supply
Farmington, MO

Title of report: Pharmacokinetic Study of 1,4-Dichlorobenzene (p-DCB) in the F344 rat and B6C3F1
Mouse Following Inhalation and Oral Administration

Author(s): Alan G.E. Wilson; Lori J. Hall; B. Richard Dudek; Celeste M. Reisch

Report issued: November 9, 1990

Conclusions:

The disposition and kinetics of ¹⁴C-1,4-Dichlorobenzene was investigated in male and female Fischer 344 rats and B6C3F1 mice following both oral and inhalation exposures. In rats, oral exposures were conducted at single doses of 149 and 305 mg/kg, and repeated oral exposure at 309 mg/kg. Inhalation exposures were conducted in male rats at 160 and 502 ppm (455 and 645 mg/kg), and in female rats at 161 and 496 ppm (308 and 678 mg/kg). In mice, single oral exposures of 310 and 638 mg/kg were conducted, as were inhalation exposures at 158 and 501 ppm (631 and 1240 mg/kg). Intravenous dosing was performed in male rats at doses of 216 and 217 mg/kg.

1,4-dichlorobenzene was rapidly but incompletely absorbed after oral and inhalation administration. Absorption after inhalation exposure was poor in comparison to oral exposure, but mice demonstrated increased absorption relative to rats after both oral and inhalation exposure. Significant tissue distribution was observed in the kidney, liver, fat, and residual carcass of dosed rats and mice. Excretion was relatively rapid at all doses tested, with a majority of radioactivity eliminated in the urine and feces by 48 hours. No biologically significant sex-related differences in excretion were noted. Repeated oral dosing did not significantly alter the disposition of 1,4-dichlorobenzene in male rats. Potential accumulation of 1,4-dichlorobenzene in tissues is suggested from reported beta elimination half lives.

Fecal elimination of ^{14}C -1,4-dichlorobenzene derived radioactivity was apparently the result of unabsorbed test chemical, although definitive proof was not provided. Enterohepatic recirculation of 1,4-dichlorobenzene has been previously demonstrated.

Urinary metabolites of ^{14}C -1,4-dichlorobenzene were isolated and tentatively identified by reversed phase HPLC with radiochemical detection. Major metabolites reported were the sulfate and glucuronide conjugates of the oxidative product 2,5-dichlorophenol. In rats, the sulfate conjugate of 2,5-dichlorophenol was the major metabolite identified from rats exposed orally or by inhalation. Induction of glucuronidation appeared to occur after repeated oral but not inhalation exposure in male rats. Tissue clearance half life was also reduced by repeated oral and inhalation exposure in male rats, as well as in female rats exposed at the high inhalation concentration of 1,4-dichlorobenzene. Clearance kinetics were largely bi-exponential, with the exception of the male rat kidney, which was found to be mono-exponential after oral exposure but not inhalation exposure. Differences in disposition and biotransformation of 1,4-dichlorobenzene from oral or inhalation exposure, with the exception of percent absorption, were minor and were not considered biologically significant.

Core Classification: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats.

Resolution of the following items is required in order to upgrade this study to core minimum data:

1) It is recognized that certain chemicals result in renal tumorigenesis in male rats through initiation of a sequence of events involving alpha-2 μ -globulin. It is further recognized that this association is unique to male rats and that risk assessment to determine the carcinogenic hazard of a chemical in humans may exclude this type of data. It is apparent that such a relationship may exist for 1,4-dichlorobenzene in male rats. However, results of the NTP 2-year bioassay show increased incidence of hepatocellular carcinoma and adenoma in male and female mice. The registrant is asked to address the question as to whether the mechanism of hepatic tumorigenesis in mice may bear any similarity to that in rats with respect to reactive metabolites produced in both species. Specifically, the methyl sulfoxide and methyl sulfone metabolites of 2,5-dichlorophenol were not addressed in this study and may be associated in some way with the mechanism(s) of hepatic and/or renal tumorigenesis. Representative chromatograms from rats and mice, as illustrated on pages 258-259 of the report, indicate several similarities despite the lack of data on this subject.

2) Clarification of kidney $t_{1/2}$ alpha as defined for male rat kidney clearance after oral exposure. If clearance is mono-exponential, then this term should actually refer to elimination and not distribution, as the term "alpha" implies.

3) Distribution and elimination curves generated for tissues in this study are requested. It appears that only two time points each for distribution and elimination were used to estimate half lives for tissues in this study. This leads to the question as to the goodness of fit for the experimental data, if only two time points each were used to estimate distribution and elimination half-lives.

4) If cumulative percent of dose is illustrated in Table 20, page 105 of the report, explain the apparent decrease observed in percent excretion between 3 and 7 days for group 9 (multiple oral dose).

5) The registrant is asked to address the issue of radiolabel stability as it applies to the preparation and use of dosing solutions. If, as stated in the report, radiolabel purity was observed to decline over the course of this study, then dose solutions used may have contained radiolabel with significant amounts of impurities. These impurities may have the potential to interfere with detection and/or identification of urinary metabolites.

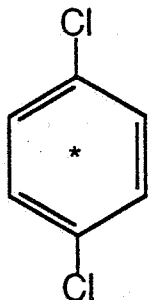
6) The registrant is asked to explain the missing urinary radioactivity from male rats dosed singly with 150 and 300 mg/kg, as mentioned on page 18 of this review.

I. MATERIALS

A. Test Material

- 1) [U-¹⁴C]1,4-dichlorobenzene (lot # CSL-87-141-71-35)
obtained from Chemsyn Science Laboratories
Radiochemical Purity: > 99.0%
Specific Activity: 55.7 mCi/mg
Note: A decrease in radiochemical purity was noted in the latter phase of the study (page 249); purity was decreased from approximately 98.0% to 86.6% at the end of the study.
- 2) Unlabeled 1,4-dichlorobenzene
lot # : not stated
Chemical Purity: > 99.0%
Unlabeled test material stated as stable (page 248).

Structure:



Metabolite Characterization and Identification

Urinary metabolites of 1,4-DCB were characterized using reversed phase gradient HPLC. Other than identification of glucuronide and sulfate conjugates by incubation with glucuronidase/sulfatase, the method of identification of other urinary metabolites (mass spec, comparison to known standards) was not stated.

B. Vehicles: Laboratory grade corn oil (oral dosing); Emulphor:ethanol (1:1; intravenous dosing); no vehicle for inhalation exposures.

C. Test Animals: Species: rat and mouse
Strain: Male and female F344 rat; Male B6C3F1 mouse.

Source: Charles River, Wilmington, MA

Weights: **Rats:** males, 142-213g (mean: 178.4g); females, 163-174g (mean: 168.6g). Age: male rats, 7-11 weeks; female rats, 13 weeks (according to study protocol, page 310 of the report).

Mice: males only; 22-30 (mean: 25.5g). Age: 7-11 weeks (according to study protocol, page 310 of the report).

II. METHODS

A. Study Design

1) Metabolism

The disposition and pharmacokinetics of ^{14}C -1,4-dichlorobenzene was assessed in male and female rats and male mice following oral, inhalation, and intravenous administration of the test compound. In rats, oral exposures were conducted at single doses of 149 and 305 mg/kg, and repeated oral exposure at 309 mg/kg. Inhalation exposures were conducted in male rats at 160 and 502 ppm (455 and 645 mg/kg), and in female rats at 161 and 496 ppm (308 and 678 mg/kg). In mice, single oral exposures of 310 and 638 mg/kg were conducted, as were inhalation exposures at 158 and 501 ppm (631 and 1240 mg/kg). Intravenous dosing was performed in male rats at doses of 216 and 217 mg/kg. Dosing procedures for each route of exposure were as follows:

a) Oral Dosing:

Test material was dissolved in laboratory grade corn oil and the dpm per gram of each dosing solution was determined by liquid scintillation counting of dose solution aliquots. Rats and mice were dosed using straight Perfektum[®] 16 gauge 2-inch (rats) or 1.5-inch (mice) feeding needles fitted to a 1ml glass tuberculin syringe. Dose administered was determined by weighing the syringe prior to and following dosing.

b) Intravenous Dosing:

Test material was dissolved in emulphor : ethanol (1:1) to allow the proper dose to be delivered to each animal in a volume of 0.1-0.3 ml of vehicle. Rats were dosed using a 25-gauge needle connected to a 1ml glass tuberculin syringe. Dose administered was determined by weighing of the glass syringe prior to and following dosing.

c) Inhalation exposure:

Exposure to test material was conducted within a closed loop exposure/monitoring system (Figure 1, page 236 of the report, attached). In this system, radiolabeled test material was

solvated in methylene chloride and added to unlabeled test material in the inhalation chamber generator bulb. The methylene chloride was allowed to evaporate and a homogeneous mixture obtained by melting of the crystals under gentle heat. This preparation was then stored frozen until use.

Animals were exposed nose-only in this inhalation exposure system. A Miran IR gas analyzer was used to monitor test material concentration. Three of the plastic tubes into which animals were inserted for inhalation exposure were modified to serve as plethysmographs from which minute volumes were calculated. Mean minute volume determined from 3 animals was used as representative of the entire group for calculating inhaled dose for individual animals. Concentration of test chemical and minute volume determinations were made approximately every 30 minutes. Inhaled dose was calculated based upon the following formula:

$$\frac{\text{Chamber Concentration (mg/L)} \times \text{Minute Volume (L/min)} \times \text{Time (min)}}{\text{Body Weight (kg)}}$$

All inhalation exposures were for 360 minutes (6 hours) for both the single and repeated dose exposures. It was not stated whether an equilibration period was necessary for the inhalation exposure system before exposure of the animals to test material.

2) Dosing Data: A comparison of theoretical vs actual doses administered for each dose group is attached to this review (Table 2, page 79 of the report). Examination of these data revealed that the majority of dose groups were within acceptable (13%) limits of the targeted dose level. The following dose groups and percent of nominal dose were found to occur with larger deviations from nominal:

- Group 1 (rat, i.v. dose of 150 mg/kg)- 144% of nominal dose
- Group 2 (rat, oral dose of 150 mg/kg)- 144% of nominal dose
- Group 5 (rat, inhalation exposure of 160 ppm)- 151% of nominal dose
- Group 10 (rat, repeat inhalation exposure of 500 ppm)- 145% of nominal dose

The number of animals assigned to each dose group and the purposes for which they were used is attached to this review (Table 3, page 80 of the report).

Dose levels for the oral portion of this study were selected based upon doses used in the NTP study on 1,4-dichlorobenzene. Inhalation exposures were selected to reflect an equivalent oral exposure dose as determined in a pre-study workplan conducted by the performing laboratory (EHL # 88043).

2) Metabolite Characterization and Identification

Metabolites of 1,4-dichlorobenzene were determined **only** in urine from dosed animals in groups 3 through 14. Urine samples for a given time period (6, 24, 48, and 72 hours and 4-7

days) were pooled for all the animals within that group. Metabolites were separated using reversed phase gradient HPLC with radiochemical detection. Metabolite peaks were reported as area percents of recovered activities. Specific procedures for identification of urinary metabolites were not presented.

C. Experimental

a. Animal Husbandry

Animals were acclimated to the laboratory environment for at least 10 days before dosing. Animals used for inhalation studies were acclimated to the inhalation exposure system for a minimum of 2 hours per day for 2 days. Following exposures, animals were housed in either stainless steel metabolism cages or in Roth metabolism cages. Animals were given food (Purina Certified Rodent Chow 5002) and water *ad libitum* except during inhalation exposures. This assumes then that food was available to orally dosed animals at all times. The conditions under which animals were housed were not stated in the methods section or study protocol section of the report.

b. Dosing

Dosing procedures for each dose group are described above in this review. As mentioned, the stability of the radiolabel appeared to be in question over the study period. A decline from approximately 98.0% to 86.6% in radiochemical purity was reported for the period of this study (page 249 of the report). The registrant stated (page 3) that determination of stability of test material in dosing vehicles was not performed due to the fact that dose solutions were prepared a short time before dosing. However, the use of radiolabel which declined in purity over time could result in the use of dose solutions for some groups (repeated oral dosing, for example) which are not pure. Thus, peaks representing chemical impurities could interfere with urinary metabolite identification if the radiolabel decomposes over time. In addition, stability of test article derived radioactivity in urine samples may also be questioned if radiolabel itself is not stable.

The quantity of radioactivity received by each rat was provided (page 40 of the report).

c. Sample Collection and Analysis

Rats were placed in individual steel or glass metabolism cages for sample collection during the study. Urine and feces were collected from animals in dose groups 3-14 and total weights determined daily until study termination. Cages were rinsed with water at each collection period and the rinse was allowed to mix with the urine sample. Collection times for urine and feces were 6, 24, 48, and 72 hours, and 4-7 days. It was stated that all samples were stored frozen until analysis. The conditions under which samples were obtained is assumed to be room temperature.

Expired air was collected from rats in the single oral dose groups of 150 and 300 mg/kg

(groups 4 and 9), as well as from mice in the single oral dose group of 600 mg/kg (group 12). Air was passed through 3 charcoal traps followed by 2 CO₂ traps (ethanolamine in methoxyethanol, 5M). The first charcoal trap and both CO₂ traps were exchanged every 24 hours, while the remaining 2 charcoal traps were kept in place for the entire collection period.

Blood samples for kinetic analysis were collected from rats in groups 1 and 2 (i.v and oral doses of 150 mg/kg) *via* the tail vein into a Vacutainer[®] tube with 500 U lithium heparin. Blood samples were stored at 4 °C following centrifugation (when volume permitted separation of plasma and cellular fractions).

At the end of the 7 day collection period following dosing, rats were killed by CO₂ asphyxiation and a blood sample obtained from the abdominal aorta. The liver, kidney, muscle, lung, and fat were removed, washed in isotonic saline, and frozen at -20 °C until analysis. Residual carcass was also stored frozen until analysis for 1,4-dichlorobenzene derived radioactivity.

Residual tissue and carcass radioactivity was analyzed through combustion of relevant samples and liquid scintillation counting. Aliquots of whole blood were analyzed by solubilization and liquid scintillation counting, while plasma was counted directly. A modified procedure was used to analyze for residual carcass radioactivity from animals exposed by inhalation. This procedure was necessary due to the high variance observed in carcass replicates which apparently resulted from radioactivity on animal fur.

Aqueous samples (urine, cage washes, CO₂ trap) were analyzed in duplicate by aliquoting into 15ml Ultimagold. Fecal samples were first homogenized in water before counting. Radioactivity in charcoal traps was flushed out with methylene chloride, and aliquots of this solution were counted. In addition, the charcoal itself was combusted to account for any remaining radioactivity.

In addition to tissue and carcass analysis for residual radioactivity, whole body autoradiography was performed on 2 animals / group from groups 4, 6, 8, 9, 10, 12, and 14.

d. Pharmacokinetic Analysis

A modified Gauss-Newton method (EXPFIT) was employed for non-linear regression analysis of pharmacokinetic data obtained from dosed animals. Data from individual animals were fitted to the equation $C = Ae^{-at} + Be^{-bt}$, where C= the concentration of 1,4-dichlorobenzene at time "t", and A and B are the intercept values obtained from "a" and "b", the first-order elimination rate constants. Half life for the alpha and beta phases was calculated according to the equation $t_{1/2} = \frac{\ln 2}{k_e}$

k_e

e. Statistical Analysis

According to the report, some data calculations were performed using RS/1 statistical software. Tests for statistical significance were performed using the t-test on RS/1.

D. Compliance

A signed statement of no data confidentiality claims was provided.

A signed statement of GLP compliance (40 CFR 160.35) was provided.

A signed statement of quality assurance was provided.

III. RESULTS

A) Disposition Summary

1. Absorption

Absorption of 1,4-dichlorobenzene was summarized by the registrant in Table 78, page 208 of the report. According to these data, percent oral absorption ranged from 62% in orally dosed rats given repeated doses at the nominal 300 mg/kg dose level to 72% in singly dosed rats at the 300 mg/kg nominal dose level. In male mice, 71% absorption was calculated from singly dosed mice (600 mg/kg nominal dose level).

Inhalation exposure resulted in significantly lower percent absorption in rats given single inhalation exposures of 160 ppm (33%) and multiple exposure of 500 ppm (25%). In mice, absorption appeared higher in exposed mice at the 160 ppm dose level (59%), but the reason for this difference was not made clear from available data. Results of measurement of inhalation parameters (pages 215-244 of the report) indicate that respiratory depression occurred in both rats and mice exposed to test chemical by inhalation, and that this decrease in respiration was dose-related. This would be expected to result in decreased absorption for both species; however, the decrease in mice was not as great as that observed in rats, as discussed above.

2. Elimination

The disposition of 1,4-dichlorobenzene in rats and mice following oral and inhalation exposures is summarized below in the following tables. Significant differences, where either statistically or biologically significant, are discussed in relation to the summarized data.

Table 1a

Excretion of [^{14}C]-1,4-dichlorobenzene Derived Radioactivity in Male Rats and Mice Following Oral Exposure^a

	<u>LDMR</u>	<u>HDMR</u>	<u>RDMR</u>	<u>LDMM</u>	<u>HDMM</u>
urine	59.83±4.21	55.80±4.18	48.95±18.22	72.35±4.65	61.10±9.30
feces	8.03±0.29	12.50±0.68**	12.87±0.67	11.11±0.38	15.74±2.72
expired air	ND	12.44±0.88	10.06±0.87	ND	9.08±0.40
carcass+ tissues	1.39±0.14	3.94±0.73	2.77±1.09	0.49±0.04	0.54±0.06
cage wash	11.51±1.01	14.40±2.94	7.90±1.53	2.23±0.40	5.27±2.95
Total	80.76±4.80	99.08	82.55	86.18	91.73

a- data taken from pages 93, 105, and 150 of the report, N=3. * p < 0.05; ** p < 0.01. Abbreviations used: LDMR, low dose male rats (150 mg/kg); HDMR, high dose male rats (300 mg/kg); RDMR, repeat dose male rats (300 mg/kg x 14 days); LDMM, low dose male mice (300 mg/kg); HDMM, high dose male mice (600 mg/kg); ND, not determined. Values are expressed as mean cumulative percent.

Table 1b

Excretion of [^{14}C]-1,4-dichlorobenzene Derived Radioactivity in Male and Female Rats Following Inhalation Exposure^a

	<u>LDMR</u>	<u>HDMR</u>	<u>LDFR</u>	<u>HDFR</u>	<u>RDMR</u>
urine	25.01±1.63	32.14±2.22	23.14±0.90	30.37±3.19	18.32±0.77**
feces	2.48±0.46	3.27±0.52	1.94±0.36	2.59±0.17	2.09±0.22
expired air	ND	ND	ND	ND	ND
carcass+ tissues	5.90±0.94	4.16±0.70	5.90±0.99	4.55±0.84	2.60±0.48
cage wash	2.91±0.20	2.86±0.30	1.64±0.35	3.82±1.40	3.82±0.32
Total	36.31±1.76	42.42±3.01	32.62±1.08	41.33±1.38**	26.83±0.70**

Table 1b, continued

a- data taken from pages 115, 128, and 137 of the report, N=3. Abbreviations are: LDMR, low dose male rats (160 ppm); HDMR, high dose male rats (500 ppm); LDFR, low dose female rats (160 ppm); HDFR, high dose female rats (500 ppm); RDMR, repeat dose male rats (500 ppm x 14 days); ND, not determined. Values represent the mean cumulative percent dose excreted.
* p < 0.05; ** p < 0.01.

Table 1c

Excretion of [¹⁴C]-1,4-dichlorobenzene Derived Radioactivity in Male Mice
Following Inhalation Exposure^a

	LDMM	HDMM
urine	32.40±6.96	47.84±3.20
feces	19.18±10.35	6.08±1.76
expired air	ND	ND
carcass+ tissues	1.14±0.24	4.03±0.59
cage wash	1.22±0.13	1.54±0.07**
Total	53.93±3.13	59.49±5.45

^adata taken from page 162 of the report, N=3. Abbreviations are: LDMM, low dose male mice (160 ppm); HDMM, high dose male mice (500 ppm); ND, not determined. Values represent mean cumulative percent excretion.

a) Oral Exposure

Oral administration of 1,4-dichlorobenzene to rats resulted in 55-59% of an administered dose excreted in urine by male rats (Table 1a). Dose of chemical did not significantly affect the percentage excreted by the urinary route. Repeated oral dosing at the 300 mg/kg nominal dose level resulted in less excretion through the urine (48%), but this difference was not significant. In general, residual carcass + tissue levels were slightly higher in animals exposed to the 300 mg/kg nominal dose (2-4%) than in animals exposed to the 150 mg/kg nominal dose (1.3%). Expired air accounted for between 10-12% of an administered dose in animals exposed singly or repeatedly to the 300 mg/kg nominal dose. Percent excretion through expired air at the 150

mg/kg nominal dose was not measured. If this percentage was found to be lower than that observed at 300 mg/kg, an increase in the percent excreted at the 300 mg/kg nominal dose level would have indicated saturation of biotransformation. However, data were not generated for comparison.

In mice exposed orally, urinary excretion appeared greater (61-72% at the 600 and 300 mg/kg nominal doses, respectively), but were likely the same as rats, as the percentage recovered from cage washes was greater in rats (11-14%) and gives equivalent percentages excreted in urine when this is added to the urinary percent in rats. Percent of administered dose recovered in feces and expired air was similar in mice as that found in rats. The percent of administered dose found in the carcass + tissues was much less in mice (0.5%) than in rats.

b) Inhalation Exposure

In male and female rats exposed through inhalation of test chemical at 160 ppm, urine was found to be the major route of elimination as for oral exposure. However, the percentage recovered was significantly less than in oral exposures (approximately 25% recovered in urine by this route). A slight increase (approximately 7%) was observed in urinary excretion at the 500 ppm nominal dose. Repeated inhalation exposure at 500 ppm resulted in significantly less urinary radioactivity in male rats (18%). Fecal excretion was also found to be diminished in all rats exposed by inhalation when compared to orally exposed rats (Table 1b). The percentage reported in carcass + tissues for rats exposed by inhalation was slightly greater (4-5%) than that reported for orally exposed rats (1-4%). This could be due to residual radioactivity present on the pelt of the animal, but tissue residues have not yet been examined in this review. Total recovery in rats exposed by inhalation was poor (32-42%) in relation to orally exposed rats. An explanation was not provided, but could be due to volatilization of test material during animal processing, or as indicated in Table 78, page 208, to decreased absorption of test chemical by the inhalation route. Absorption for orally exposed rats and mice ranged between 62-72%, but was calculated by the registrant as only 25-59% for rats and mice exposed by inhalation.

c) Time Course of Elimination

Examination of the Tables from which the above data on terminal disposition were obtained also gives an indication as to the rapidity with which administered radioactivity was excreted in rats and mice. These Tables are : Table 14, page 93; Table 20, page 105; Table 25, page 115; Table 31, page 128; Table 36, page 137; Table 42, page 150; and Table 48, page 162 of the report. These are attached to this review for reference in the following summary.

In orally dosed rats, between 6-11% of the administered dose was excreted by 6 hours in singly and repeatedly dosed rats at the 150 and 300 mg/kg nominal dose levels. Approximately 6% of an administered dose was excreted in rats exposed by inhalation (Table 25) by 6 hours. In rats dosed by the oral route as well as by inhalation, the majority of urinary radioactivity was excreted by 24 hours. In female rats exposed by inhalation, only 1-3% of an administered dose was excreted in the urine by 6 hours, and excretion in urine did not appear complete until 72 hours (Table 36, page 137 of the report).

In orally exposed mice, a similar pattern of excretion for urine was reported, in that the majority of radioactivity was excreted by this route in 24 hours. However, by 6 hours, orally dosed mice had excreted greater than 2 times the percentage excreted by orally dosed rats within this time (Table 42, page 150). In contrast to rats, increasing the oral or inhaled dose in mice resulted in a substantial increase in the percentage excreted through the urine in the first 6 hours following exposure (Tables 42 and 48, pages 150 and 162 of the report).

While fecal excretion was largely complete in all dose groups by 24 hours, in female rats and male mice exposed by inhalation, fecal excretion appeared to increase with time during the 7 day period (Tables 36 and 48, pages 137 and 162 of the report).

3. Distribution

Tissue distribution data showing the time course of distribution for radioactivity, the tissue / blood ratio, and percent of administered dose were provided in Tables 15, 16, 21, 26, 27, 32, 37, 38, 43, 44, 49, and 50 (attached to this review).

It is possible to draw several general conclusions regarding these data:

- i) Highest levels of tissue radioactivity were observed in the kidney, liver, fat, and carcass of both rats and mice in all treatment groups.
- ii) Tissue levels of radioactivity appeared to decline fairly rapidly. Less than 0.5% of an administered dose was reported at 3 days post-exposure for any tissue examined, with the exception of the carcass of rats after inhalation exposure (Tables 26, 27, and 32). This could be due to residual radioactivity on the pelt of the rats.
- iii) The highest tissue/blood ratios (greater than 1) were observed in the kidney, fat, and carcass of all treated groups.
- iv) Elimination of 1,4-dichlorobenzene derived radioactivity appeared slower in the kidneys of dosed male rats vs female rats or male mice. This is supported by the increased tissue half-life observed for kidney in male rats from all dose groups (Tables 18, 23, 29, and 32) when compared to other analyzed tissues. The basis for increased half-life of 1,4-dichlorobenzene in male rat kidney is apparently associated with the presence of alpha-2- μ -globulin, which is known to bind with 1,4-dichlorobenzene and its metabolite, dichlorophenol.
- v) Tissue levels of radioactivity appeared to increase proportionately with dose in most cases, indicating a first-order elimination process. This is also supported by the normalized tissue concentration data, which illustrates that tissue levels of radioactivity were constant on a per mg/kg basis at both low and high doses. However, accumulation in the kidney is suggested from increased normalized tissue values observed in male rats exposed repeatedly by oral gavage or inhalation. Values for fat, in contrast, were observed to decline in male rats exposed repeatedly to test chemical.

4. Plasma Levels of ^{14}C 1,4-dichlorobenzene derived Radioactivity

The most extensive data provided for blood levels of radioactivity were shown in Tables 12 and 13, pages 90-91 of the report. Table 12 presented results of serial blood sampling in male rats dosed intravenously with 217 mg/kg 1,4-dichlorobenzene, and compares these data to male rats dosed orally with 216 mg/kg 1,4-dichlorobenzene. Table 13 shows derived alpha and beta half-lives from the intravenously and orally dosed rats.

Graphical and tabular representation of blood level data are made below:

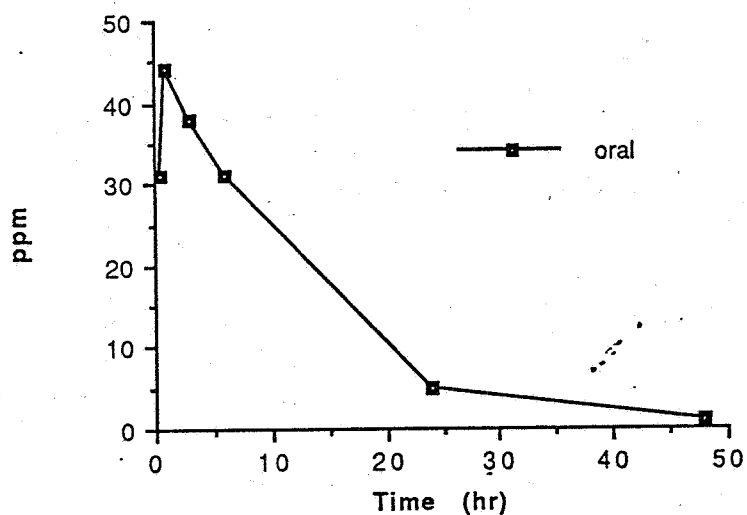
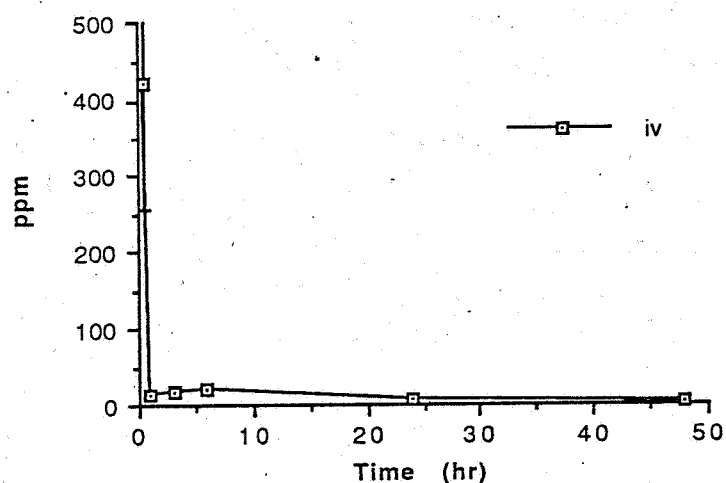


Table 2

Blood Levels of 1,4-Dichlorobenzene Following Oral or Intravenous Administration in Male F344 Rats^a

Time (hr)	<u>I.V. 217 mg/kg</u>	<u>Oral 216 mg/kg</u>
0.5	422±166	31±16
1.0	14±2	44±3
3.0	16±1	38±2
6.0	18±2	31±0.5
24	5.2±0.6	4.9±0.5
48	2.3±0.2	1.0±0.1

^adata from Table 12, page 90 of the report.

According to the registrant, the decline in blood levels followed an apparent bi-exponential process for both i.v. and orally dosed rats. Distribution half life was approximately 4 minutes in intravenously dosed rats and 3.5 hours in orally dosed rats. Elimination half life was stated as 0.67 and 0.46 days following intravenous and oral administration, respectively.

B. Whole Body Autoradiography

Two animals each from dose groups 4 (rat, male, single oral 300 mg/kg), 6 (rat, male, single inhalation 500 ppm), 8 (rat, female, single inhalation 500 ppm), 9 (rat, male, repeat oral 300 mg/kg), 10 (rat, male, repeat inhalation 500 ppm), 12 (mouse, single oral 600 mg/kg), and 14 (mouse, single inhalation 500 ppm) were selected for use in determination of whole-body distribution of 1,4-dichlorobenzene derived radioactivity. It was not stated at what time following dosing that animals were sacrificed for this procedure, except in male rats from group 4, which were sacrificed 1 day after gavage treatment.

Results of this portion of the study were presented as xeroxed photographs of animals subjected to autoradiography. Examination of these photographs showed that localization of radioactivity was similar among rats and mice exposed either orally or by inhalation, and included significant concentrations of 1,4-dichlorobenzene derived radioactivity in nasolacrimal duct, urethra, preputial gland, and gut and stomach contents. In rats, the kidney also contained significant amounts of 1,4-dichlorobenzene derived radioactivity, which was not observed in

mice. The radioactivity observed in the fat of mice also appeared to be of a different nature than that observed in rats, as radioactivity in fat of rats only appeared in frozen sections (indicating volatile material), whereas radioactivity in fat of mice appeared in dehydrated sections, indicating non-volatile material.

2) Metabolite Characterization and Identification

According to information supplied in the report (pages 251-252), urinary metabolites of 1,4-dichlorobenzene were characterized by comparison of retention times as well as comparison of urinary profiles before and after incubation with sulfatase/glucuronidase. No other types of analyses were involved in metabolite characterization.

According to the data presented, three radiochemical peaks were characterized in urine of all treated groups. These were reported as the glucuronide and sulfate conjugates of 2,5-dichlorophenol, and free 2,5-dichlorophenol. Other peaks were present in radiochromatograms and were suggested to be the sulfoxide and sulfone metabolites of 2,5-dichlorophenol, but these were not definitively identified. As stated, these peaks individually represented less than 5% of the administered dose, but collectively, accounted for between 5-21% of the dose (page 25 of the report).

Summary of identified metabolites was made in Tables 19, 24, 30, 35, 41, 47, and 53 of the report. A summary Table illustrating the cumulative percent of each metabolite identified in urine of the various dose groups at 7 days is shown below.

Table 4
Urinary Metabolites of 1,4-Dichlorobenzene in Male and Female
F344 rats Exposed by Oral Gavage or Inhalation^a

	<u>Metabolite (Cumulative % Dose)</u>		
	<u>Glucuronide</u>	<u>Sulfate</u>	<u>2,5-Dichlorophenol</u>
<u>Rats</u>			
Male oral 150 mg/kg	6.4	28.0	1.65
Male oral 300 mg/kg	5.9	30.6	4.45
Male oral 300 mg/kg x 14 days ^b	16.4	29.9	2.87
Male inhal. 160 ppm ^c	1.9	19.4	2.49
Male inhal. 500 ppm ^c	6.1	21.3	0.66

Table 4, continued
Metabolite (Cumulative % Dose)

	<u>Glucuronide</u>	<u>Sulfate</u>	<u>2,5-Dichlorophenol</u>
Male inhal. 500 ppm x 14 days ^c	6.3	12.4	1.15
Female inhal. 160 ppm	2.5	14.9	1.30
Female inhal. 500 ppm	4.2	22.2	1.75
<u>Mouse</u>			
Male oral 300 mg/kg ^d	27.3	25.2	9.10
Male oral 600 mg/kg ^c	38.6	26.9	5.82
Male inhal. 160 ppm	21.0	16.3	0.27
Male inhal. 500 ppm ^b	28.9	14.7	2.97

^adata taken from pages 104, 114, 127, 136, 148, 161, and 173 of the report. Data represents analysis of 4-7 day samples.

^bdata represents 72 hour analysis.

^cdata represents 48 hour analysis.

^d data represents 24 hour analysis.

In rats exposed either orally or by inhalation, the sulfate conjugate of 2,5-dichlorophenol was reported as the major urinary metabolite (Table 4, above). Repeated oral exposure of rats to the 300 mg/kg nominal dose resulted in apparent induction of glucuronidation, as shown by the increased amount of 2,5-dichlorophenol glucuronide and the decreased amount of free 2,5-dichlorophenol. Inhalation exposure did not produce a similar effect, but as absorption from inhalation exposure was low (25-33%), the apparent induction may not occur, as plasma levels of test chemical would be lower in rats exposed by inhalation. This hypothesis was not tested in the present study, but is supported in part by the similar pattern of urinary metabolites observed between rats in the low oral dose group and the high inhalation exposure group.

Female rats were exposed only by the inhalation route, but the pattern of glucuronide and sulfate conjugates produced, as well as free 2,5-dichlorophenol, appear similar to that of male rats similarly exposed. It is noted that an orally exposed female rat group would have been useful for comparison of metabolism between male and female rats. This is discussed further below.

Mice, in contrast to rats, showed an approximate equivalence in the production of glucuronide and sulfate conjugates in urine. Apparent induction of glucuronidation was also observed between mice of the 600 vs 300 mg/kg oral dose groups. In mice exposed by the inhalation route, some apparent induction of glucuronidation was also evident; however, the amount of free 2,5-dichlorophenol was also increased, suggesting that the kinetics of production of this metabolite may be different in mice.

Identification of additional metabolites was not made in this study. It was suggested that the additional peaks observed were the sulfoxide and sulfone metabolites of 2,5-dichlorophenol, which can be considered as potentially more reactive towards tissue macromolecules than 2,5-dichlorophenol. The reactive nature of these metabolites may underly the mechanism(s) of hepatic and/or renal tumorigenesis as reported in the NTP bioassay on 1,4-dichlorobenzene.

Examination of metabolite recovery data (pages 262-263 of the report) shows that for most dose groups, recovery of urine as various metabolites agreed well with total urinary radioactivity (Tables 1a-c above). However, for male rats dosed singly with nominal oral doses of 150 and 300 mg/kg, 23.8% and 14.9% of urinary radioactivity was unaccounted for in these dose groups, respectively.

IV. DISCUSSION

Data were presented in this study demonstrating the effects of species, route of administration, and dose on the disposition and metabolism of radiolabeled 1,4-dichlorobenzene. Examination of disposition data (Tables 1a-1c, above) show that in general, the routes of elimination and the percentage of an administered dose of 1,4-dichlorobenzene eliminated are similar among rats and mice. Inhalation exposure resulted in less total recovery of radiolabel from the various routes of elimination, but qualitatively, the routes of elimination were comparable to oral exposure. It should be noted that absorption in both rats and mice exposed via inhalation was poor in comparison to oral absorption of test material (33%, 25%, and 59% for male rats exposed singly at 160 ppm, exposed multiply at 500 ppm, and mice exposed singly at 160 ppm, respectively). Thus, the actual doses of test material received by these dose groups would be nominal doses of **52.8, 125, and 94.4 ppm**, respectively. While it is evident that absorption in rats and mice exposed by inhalation was poor in relation to orally exposed animals, absorption for rats exposed to the 300 ppm nominal dose for 14 days may be inaccurate, as multiple exposure could alter absorption. For both oral and inhalation exposure, mice demonstrated increased absorption in relation to rats.

Distribution of 1,4-dichlorobenzene derived radioactivity was found to occur in significant quantity to the kidney, fat, and residual carcass. Accumulation (tissue/blood ratio greater than 1) was observed in kidney, liver, fat, carcass, and plasma of dosed animals. Elimination from the kidney of male rats appeared slower, as supported by increased beta elimination half-life for this organ as compared to other organs examined (except blood). According to the registrant, elimination of 1,4-dichlorobenzene derived radioactivity was mono-exponential in orally dosed rats, whereas bi-exponential elimination was observed in rats exposed via inhalation (page 48 of the report). It is possible (but not proven) that the altered kidney elimination kinetics is based upon binding of test chemical and/or metabolite to alpha 2 μ -globulin, found only in male rat

kidney. Although elimination of tissue radioactivity was fairly rapid (less than 0.5% present after 72 hours), the long beta elimination half lives reported for the tissues studied after single and repeated doses (attached to this review) indicate the potential for accumulation of 1,4-dichlorobenzene in tissues from repeated daily exposures.

Metabolites of 1,4-dichlorobenzene were characterized only in urine. From these data, it is apparent that there are species differences in metabolism of 1,4-dichlorobenzene. In rats, sulfate conjugation appeared to be the predominant pathway of biotransformation in rats exposed both orally and by inhalation. Apparent induction of glucuronidation was observed in rats given repeated oral exposure, but not from repeated inhalation exposure. This difference could be related to poor absorption of test chemical from inhalation exposure. In mice exposed both orally and by inhalation, a larger percentage of radioactivity was present in urine as the glucuronide conjugate of 2,5-dichlorophenol. The percentage of free 2,5-dichlorophenol appeared to decrease with increasing oral exposure in mice, while in rats, the percentage of this metabolite appeared to increase with increasing oral exposure. Inhalation exposure resulted in increasing amounts of urinary 2,5-dichlorophenol for both rats and mice. Although no direct evidence is presented, these data suggest that oxidation of 1,4-dichlorobenzene to 2,5-dichlorophenol was saturated at higher oral exposures in mice. This is supported by the greater percentage of orally absorbed 1,4-dichlorobenzene observed in mice vs. rats. Inhalation exposure, in which absorption was significantly lower, could fail to saturate this oxidative pathway in either species, resulting in the metabolite pattern for 2,5-dichlorophenol as observed in this study.

Aside from the observed differences in oxidative metabolism between rats and mice, the increased glucuronidation of 1,4-dichlorobenzene in mice suggests species differences in glucuronyltransferase activity. This is supported by previous work on benzene (Sabourin et al., *Toxicol. Appl. Pharmacol.*, 99, 421-444 (1989) in which increased production of hydroquinone glucuronide from orally administered benzene was reported in B6C3F1 mice, the same strain as used in the present work.

With the exception of the male kidney, tissue kinetics of 1,4-dichlorobenzene appeared to be biexponential (i.e., presence of a distinct alpha and beta phase). In the male kidney, elimination of 1,4-dichlorobenzene derived radioactivity was apparently mono-exponential. In addition, the length of $t_{1/2}$ alpha for male rat kidney was increased relative to other organs studied. The reason for this increased half life, as mentioned above, could be due to binding of 1,4-dichlorobenzene and/or a metabolite to alpha-2 μ -globulin, present only in male rat kidney.

The description of kidney clearance of 1,4-dichlorobenzene derived radioactivity for male rats after oral exposure (monoexponential) implies that no alpha phase was present, and that elimination would be wholly described by the beta phase. However, elimination for male rat kidney is described with the terminology $t_{1/2}$ alpha, which may be an error. Clarification from the registrant is required on this point.

Dose effects on tissue elimination kinetics appeared minor in general. Exceptions to this were found for male rats given multiple oral or inhalation exposures, and for female rats given a high inhalation exposure. In these cases, these types of exposure resulted in an apparent decrease in half life of elimination for tissues where significant radioactivity was detected (blood, fat, liver). This phenomenon was not observed in mice exposed to single doses. The decrease in apparent clearance of 1,4-dichlorobenzene derived radioactivity in male rats exposed to multiple doses is

consistent with the apparent induction of glucuronidation observed in male rats given multiple oral doses of 1,4-dichlorobenzene.

In general, it can be stated that the differences in disposition and metabolism of 1,4-dichlorobenzene as reported in this study are minor and not of major biological significance, with the exception of the large difference in percent absorption observed between orally exposed rats and mice and those exposed by inhalation. However, as it is the intention of the registrant to conduct studies by the oral route, it is apparent that oral exposure will result in much greater total exposure to test chemical than that obtained from inhalation exposure. Thus, there is no compelling reason to conduct studies via the inhalation route, as exposure through single or repeated doses does not produce a pattern of disposition and biotransformation that is significantly different than that observed from the oral route. However, it is suggested, based upon data in this study, that a range of oral doses be employed such that saturable and non-saturable metabolism of 1,4-dichlorobenzene are represented.

V. CONCLUSIONS

The disposition and kinetics of ^{14}C -1,4-Dichlorobenzene was investigated in male and female Fischer 344 rats and B6C3F1 mice following both oral and inhalation exposures. In rats, oral exposures were conducted at single doses of 149 and 305 mg/kg, and repeated oral exposure at 309 mg/kg. Inhalation exposures were conducted in male rats at 160 and 502 ppm (455 and 645 mg/kg), and in female rats at 161 and 496 ppm (308 and 678 mg/kg). In mice, single oral exposures of 310 and 638 mg/kg were conducted, as were inhalation exposures at 158 and 501 ppm (631 and 1240 mg/kg). Intravenous dosing was performed in male rats at doses of 216 and 217 mg/kg.

1,4-dichlorobenzene was rapidly but incompletely absorbed after oral and inhalation administration. Absorption after inhalation exposure was poor in comparison to oral exposure, but mice demonstrated increased absorption relative to rats after both oral and inhalation exposure. Significant tissue distribution was observed in the kidney, liver, fat, and residual carcass of dosed rats and mice. Excretion was relatively rapid at all doses tested, with a majority of radioactivity eliminated in the urine and feces by 48 hours. No biologically significant sex-related differences in excretion were noted. Repeated oral dosing did not significantly alter the disposition of 1,4-dichlorobenzene in male rats. Potential accumulation of 1,4-dichlorobenzene in tissues is suggested from reported beta elimination half lives.

Fecal elimination of ^{14}C -1,4-dichlorobenzene derived radioactivity was apparently the result of unabsorbed test chemical, although definitive proof was not provided. Enterohepatic recirculation of 1,4-dichlorobenzene has been previously demonstrated.

Urinary metabolites of ^{14}C -1,4-dichlorobenzene were isolated and tentatively identified by reversed phase HPLC with radiochemical detection. Major metabolites reported were the sulfate and glucuronide conjugates of the oxidative product 2,5-dichlorophenol. In rats, the sulfate conjugate of 2,5-dichlorophenol was the major metabolite identified from rats exposed orally or by inhalation. Induction of glucuronidation appeared to occur after repeated oral but not inhalation exposure in male rats. Tissue clearance half life was also reduced by repeated oral and inhalation exposure in male rats, as well as in female rats exposed at the high inhalation concentration of 1,4-dichlorobenzene. Clearance kinetics were largely bi-exponential, with the exception of the male rat.

kidney, which was found to be mono-exponential after oral exposure but not inhalation exposure. Differences in disposition and biotransformation of 1,4-dichlorobenzene from oral or inhalation exposure, with the exception of percent absorption, were minor and were not considered biologically significant.

VI. CLASSIFICATION: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats. Resolution of the following items is required in order to upgrade this study to core minimum data:

1) It is recognized that certain chemicals result in renal tumorigenesis in male rats through initiation of a sequence of events involving alpha-2 μ -globulin. It is further recognized that this association is unique to male rats and that risk assessment to determine the carcinogenic hazard of a chemical in humans may exclude this type of data. It is apparent that such a relationship may exist for 1,4-dichlorobenzene in male rats. However, results of the NTP 2-year bioassay show increased incidence of hepatocellular carcinoma and adenoma in male and female mice. The registrant is asked to address the question as to whether the mechanism of hepatic tumorigenesis in mice may bear any similarity to that in rats with respect to reactive metabolites produced in both species. Specifically, the methyl sulfoxide and methyl sulfone metabolites of 2,5-dichlorophenol were not addressed in this study and may be associated in some way with the mechanism(s) of hepatic and/or renal tumorigenesis. Representative chromatograms from rats and mice, as illustrated on pages 258-259 of the report, indicate several similarities despite the lack of data on this subject.

2) Clarification of kidney $t_{1/2}$ alpha as defined for male rat kidney clearance after oral exposure. If clearance is mono-exponential, then this term should actually refer to elimination and not distribution, as the term "alpha" implies.

3) Distribution and elimination curves generated for tissues in this study are requested. It appears that only two time points each for distribution and elimination were used to estimate half lives for tissues in this study. This leads to the question as to the goodness of fit for the experimental data, if only two time points each were used to estimate distribution and elimination half-lives.

4) If cumulative percent of dose is illustrated in Table 20, page 105 of the report, explain the apparent decrease observed in percent excretion between 3 and 7 days for group 9 (multiple oral dose).

5) The registrant is asked to address the issue of radiolabel stability as it applies to the preparation and use of dosing solutions. If, as stated in the report, radiolabel purity was observed to decline over the course of this study, then dose solutions used may have contained radiolabel with significant amounts of impurities. These impurities may have the potential to interfere with detection and/or identification of urinary metabolites.

6) The registrant is asked to explain the missing urinary radioactivity from male rats dosed singly with 150 and 300 mg/kg, as mentioned on page 18 of this review.

Page _____ is not included in this copy.

Pages 23 through 26 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
