TOXICOLOGY AND APPLIED PHARMACOLOGY 78, 47–54 (1985)

Disposition and Metabolism of 2,3-[^{14}C]Dichloropropene in Rats after Inhalation

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Received July 30, 1984; accepted October 23, 1984

Disposition and Metabolism of 2,3-[^{14}C]Dichloropropene in Rats after Inhalation. BOND, J. A., MEDINSKY, M. A., DUTCHER, J. S., HENDERSON, R. F., CHENG, Y. S., MEWHINNEY, J. A., AND BIRNBAUM, L. S. (1985). *Toxicol. Appl. Pharmacol.* **78,** 47–54. 2,3-Dichloropropene (2,3-DCP) is a constituent of some commercially available preplant soil fumigants for the control of plant parasitic nematodes. Human exposure potential exists during manufacture of the chemicals or during bulk handling activities. The purpose of this investigation was to determine the disposition and metabolism of 2,3-[^{14}C]DCP in rats after inhalation. Male Fischer-344 rats were exposed nose-only to a vapor concentration of 250 nmol 2,3-[^{14}C]DCP/liter air (7.5 ppm; 25°C, 620 Torr) for 6 hr. Blood samples were taken during exposure, and urine, feces, expired air, and tissues were collected for up to 65 hr after exposure. Urinary excretion was the major route of elimination of ^{14}C (55% of estimated absorbed 2,3-DCP). Half-time for elimination of ^{14}C in urine was 9.8 ± 0.05 hr ($\bar{x}$ ± SE). Half-time for elimination of ^{14}C feces (17% of absorbed 2,3-DCP) was 12.9 ± 0.14 hr ($\bar{x}$ ± SE). Approximately 1 and 3% of the estimated absorbed 2,3-[^{14}C]DCP were exhaled as either 2,3-[^{14}C]DCP or $^{14}CO_2$, respectively. Concentrations of ^{14}C in blood increased during 240 min of exposure, after which no further increases in blood concentration of ^{14}C were seen. ^{14}C was widely distributed in tissues analyzed after a 6-hr exposure of rats to 2,3-[^{14}C]DCP. Urinary bladder (150 nmol/g), nasal turbinates (125 nmol/g), kidneys (84 nmol/g), small intestine (61 nmol/g), and liver (35 nmol/g) were tissues with the highest concentrations of ^{14}C immediately after exposure. Over 90% of the ^{14}C in tissues analyzed was 2,3-DCP metabolites. Half-times for elimination of ^{14}C from tissues examined ranged from 3 to 11 hr. The data from this study indicate that after inhalation 2,3-DCP is metabolized in tissues and readily excreted. © 1985 Academic Press, Inc.

Dichloropropenes are used in combination with dichloropropane in the production of some commercially available preplant soil fumigants for the control of plant parasitic nematodes. The major constituents of these fumigants are *cis*-1,3 and *trans*-1,3 dichloropropene (1,3-DCP), which constitute nearly 60% of the total mass of the mixture (Nater and Gooskens, 1976). Smaller quantities (about 5%) of 2,3-dichloropropene (2,3-DCP) occur in these chemical mixtures. The 1978 US consumption of these fumigant mixtures has been estimated as 1.8×10^{10} g. 2,3-DCP is also an intermediate in the production of the herbicide 2-chlorallyl diethyldithiocarbamate (SRI International, 1980). Dichloropropenes are also found in oil and fat solvents and in dry cleaning and degreasing processes (Windholz, 1976). Perhaps the greatest human exposure potential exists during manufacture of the chemicals or during bulk handling activities.

Very little work on 2,3-DCP has been reported. Similar to the 1,3-DCP isomers,

0041-008X/85 $3.00

2,3-DCP is mutagenic in *Salmonella typhimurium* (De Lorenzo *et al.*, 1977; Stolzenberg and Hine, 1980; Neudecker *et al.*, 1980). Van Duuren *et al.* (1979) have demonstrated that *cis*-1,3-DCP was sarcomagenic after sc injection in mice. However, there are no data on the potential carcinogenicity of 2,3-DCP in laboratory animals. Studies (Medinsky *et al.*, 1983; Dutcher *et al.*, 1984) on the pathways of excretion of 2,3-DCP after po, ip, or inhalation administration have been reported. Oral or ip administration of 2,3-[^{14}C]DCP results in approximately 70% of the radioactivity being excreted in urine, 17% in feces, and 8% as CO_2 (Medinsky *et al.*, 1983, 1984). Following inhalation, routes of ^{14}C excretion were independent of 2,3-DCP concentration, with 50% of the ^{14}C excreted in urine, 13% in feces, and approximately 7% as CO_2 and <1% as 2,3-DCP in expired air. There has been no report on the disposition and metabolism of 2,3-DCP in tissues.

The purpose of this investigation was to determine the disposition and metabolism of 2,3-[^{14}C]DCP in rats after inhalation. Excretion and tissue distribution of 2,3-[^{14}C]DCP in rats after a 6-hr exposure to 2,3-[^{14}C]DCP are reported. In addition, uptake of ^{14}C was measured during the course of the inhalation exposure to 2,3-[^{14}C]DCP. The results from this work indicate that 2,3-DCP is rapidly metabolized in tissues and excreted primarily in the urine.

METHODS

Chemicals. 2,3-[1,3-^{14}C]Dichloro-1-propene (2,3-[^{14}C]DCP; 3.2 μCi/μmol) was obtained from Midwest Research Institute, Kansas City, Missouri. 2,3-[^{14}C]DCP was stored in tetraglyme (1 mCi/ml) at −196°C, and 0.1% of *p*-methoxyphenol was added as a free-radical inhibitor. Unlabeled 2,3-DCP (98% pure) was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin and vacuum distilled before use. The radiochemical purity of the 2,3-[^{14}C]DCP was 98% by reverse-phase high performance liquid chromatography with a 10-μm μBondapack C_{18} column (Waters Associates, Milford, Mass.) and a 10 to 100% methanol:water gradient in 30 min at a flow rate of 1 ml/min. One-minute samples of eluant were collected and counted on a liquid scintillation spectrometer (Packard Model 460CD).

Tetraethylammonium hydroxide (25% in water) was obtained from Eastman Kodak Company, Rochester, New York. Ethanolamine and 2-methoxyethanol were purchased from Fischer Scientific Company, Pittsburgh, Pennsylvania. Ready-Solv EP (liquid scintillation solution) was purchased from Beckman Instruments Company, Fullerton, California. Cab-O-Sil was purchased from Packard Instruments, Downers Grove, Illinois. T-61 (euthanasia solution; *N*-[2-(*m*-methoxy-phenyl)-2-ethyl-butyl-(1)]-γ hydroxybutyramide, 4,4′-methylene-bis(cyclohexyl-trimethylammonium iodide, and tetracaine hydrochloride) was obtained from American Hoechst Corporation, Somerville, New Jersey. All other chemicals were of the highest purity commercially available.

Animals. Male, specific pathogen-free, Fischer-344 rats (11 to 15 weeks, 200 to 250 g) were used in this study. The rats were born and raised in the Institute's barrier-maintained colony. Rats were housed two per polycarbonate cage with hardwood-chip bedding and filter caps. Animal rooms were maintained at 21 ± 2°C with a relative humidity of 20 to 50% and a 12-hr light:12-hr dark cycle, with light starting at 0600. Feed (Lab Blox, Allied Mills, Chicago, Ill.) and water from bottles with sipper tubes were provided *ad libitum* except during the inhalation exposure.

Exposure atmosphere. 2,3-[^{14}C]DCP was generated using a J-tube vapor generator (Miller *et al.*, 1980). The system consisted of a heat-taped J-tube (borosilicate glass), a temperature controller, and a syringe pump. The J-tube consisted of a column of 3-mm glass beads to increase contact between liquid 2,3-[^{14}C]DCP and nitrogen carrier gas. The carrier gas flow rate was 1 liter/min, and the dilution air flow rate was 10 liter/min of filtered air. Preliminary studies indicated that no aerosol was formed in the chamber under these conditions.

A total of 51 rats were exposed nose-only (Raabe *et al.*, 1973) for 6 hr to 250 nmol 2,3-[^{14}C]DCP vapor/liter air (7.5 ppm, 25°C, 620 Torr). All rats were exposed at the same time between 0900 and 1000. Concentration of 2,3-[^{14}C]DCP in the exposure chamber was continuously monitored by on-line infrared spectroscopy (Miran; Foxboro-Wilks, Norwald, Conn.), and radioanalysis and gas chromatographic analysis of air samples removed hourly from the exposure chamber during exposure. 2,3-[^{14}C]DCP concentration was determined by drawing a portion of exposure atmosphere (0.5 liter/min) through a cartridge of Tenax-GC (250 mg) for 5 min. The 2,3-[^{14}C]DCP was eluted from the cartridge with 5 ml pentane and quantified by liquid scintillation spectroscopy and gas chromatography (GOW-MAC series 750 FID gas chromatograph; detector—60°C; column—40°C; nitrogen carrier gas—20 ml/min). The concentration used in this study has previously been shown to be within the linear range of exposure concentrations (17 to 1650 nmol 2,3-DCP/liter air; 0.5–52 ppm, 25°C, 620 Torr)

in which absorption and excretion of inhaled 2,3-DCP were not saturated (Dutcher *et al.*, 1984).

Approach to steady-state blood concentration and tissue concentrations at steady state and after of exposure. Twenty-one rats were exposed to 2,3-[^{14}C]DCP for 10, 20, 40, and 60 min, and for 2, 4, and 6 hr. At the end of each time point, three rats were removed from the exposure chamber and immediately killed by an ip injection of 1 ml of T-61. Blood was drawn from each rat by cardiac puncture (2 ml) into heparanized vacuum tubes for analysis of ^{14}C.

Twenty-six rats were exposed to 2,3-[^{14}C]DCP for 6 hr and groups of two or three rats each were killed at 0, 0.5, 1.0, 2.0, 4.0, 8.0, 18, 24, 42, 48, and 65 hr after exposure. Rats were euthanized by ip injection of 1 ml of T-61, and the following tissues were removed for analysis of ^{14}C: liver, brain, thyroid, larynx, spleen, thymus, kidneys, adrenals, stomach (emptied of contents), small intestine sample (emptied of contents), large intestine sample (emptied of contents), urinary bladder (emptied of contents), testes, perirenal fat sample, subcutaneous fat sample, muscle sample, bone (femur), pelt sample, heart, lung, and trachea.

Pathways of excretion. Immediately after a 6-hr exposure to vapors of 2,3-[^{14}C]DCP, four rats were placed in glass metabolism cages (Stanford Glass, Palo Alto, Calif.) for collection of urine, feces, and expired air. Urine and feces were collected in glass containers on dry ice at 4, 7, 10, 15, 19, 24, 41, 48, and 65 hr after exposure. Expired air was pulled from each metabolism cage at a flow rate of 500 ml/min by a vacuum pump. The expired air passed through two cartridges, each containing 15 g of Tenax to remove 2,3-[^{14}C]DCP and then through two bubblers each containing 200 ml of 5 M ethanolamine in 2-methoxyethanol for $^{14}CO_2$ collection (Kornbrust and Bus, 1982). Samples were removed from each bubbler every hr for the first 4 hr, after which time solutions in the bubblers were sampled and changed at the times of the other excreta collections. Tenax cartridges were replaced every 24 hr. After the 65-hr collection period, the glass cages were washed with 25 ml of water to determine the amount of radioactivity remaining. After 65 hr, rats were killed by ip injection of T-61, and blood samples (2 ml) were obtained by cardiac puncture.

Excreta and tissue analysis. Samples of urine (250 μl) and ethanolamine (1 ml) from bubblers were added directly to liquid scintillation vials containing 10 ml of liquid scintillation solution for determination of ^{14}C. Tenax cartridges were each extracted with 50 ml of pentane and a 1-ml aliquot of the extract added directly to liquid scintillation vials containing 10 ml of liquid scintillation solution. Samples of feces were weighed and added to cooled vials (methanol/ice slurry) that contained 5 ml of 1% Triton X-100. The vials were capped and feces allowed to solubilize overnight.

Duplicate samples (<300 mg) of the solubilized feces were digested overnight in 1 ml of tetraethylammonium hydroxide containing 25% water. After digestion, samples were then neutralized with 1 ml of concentrated HCl, decolorized with 0.5 ml 30% H_2O_2, and allowed to sit overnight. Liquid scintillation solution (11 ml) containing 3 ml of a solution of 40 g/liter Cab-O-Sil was then added to the samples. Partially thawed carcasses were weighed, sliced into 3-cm pieces, and homogenized on ice in two vol of water. Duplicate samples of tissues and carcass (~300 mg or less) or blood (<100 μl) were placed in scintillation vials containing 1 ml of tetraethylammonium hydroxide. The vials were capped and allowed to digest overnight. After digestion, the vials were cooled (methanol/ice slurry), and 0.5 ml of concentrated HCl and 0.5 ml of 30% H_2O_2 were added to neutralize and decolorize the samples, respectively. Liquid scintillation solution (11 ml) containing 3 ml of a solution of 40 g/liter Cab-O-Sil was then added to the vials.

All samples (urine, feces, expired air, tissues, and carcass) were counted in a Packard Model 460C liquid scintillation spectrometer, with sufficient counts accumulated to give <5% error with a 95% confidence interval. Quench correction was determined using the automatic external standard method.

Quantitation of 2,3-[^{14}C]DCP metabolites in excreta and tissues. Portions of urine, feces, and tissues (including blood) in which there was sufficient ^{14}C (>1000 dpm/g) were analyzed to determine the portion of ^{14}C that was due to 2,3-DCP or metabolites. Tissue samples were weighed and homogenized in 2 vol of ice-cold distilled water. One-half milliliter of this homogenate was removed and added to 2 ml of ice-cold acetonitrile. The sample was then shaken and centrifuged (1000*g*), and the supernatant fraction was removed and placed in an ice-cold sealed glass container. The pellet was resuspended in acetonitrile and extracted twice more with acetonitrile. Preliminary work using homogenized tissues spiked with known quantities of 2,3-[^{14}C]DCP indicated that more than 95% of the 2,3-[^{14}C]DCP was extracted by this method. The supernatant fractions from the three extractions were pooled and diluted with distilled water to a final concentration of 50% water:50% acetonitrile. This mixture was then placed onto a reverse phase Sep-Pak cartridge (C_{18}; Waters Assoc., Milford, Mass.), and the eluant was collected directly into liquid scintillation vials. Preliminary studies verified that more than 98% of 2,3-[^{14}C]DCP added to a mixture of 50% water:50% acetonitrile was retained on the Sep Pak cartridge. Two milliliters of ice-cold acetonitrile was then placed onto the Sep Pak cartridge to elute the 2,3-[^{14}C]DCP, and the eluant was collected directly into a separate liquid scintillation vial. Radioactivity in the eluant consisting of 50% water:50% acetonitrile was considered to be 2,3-DCP metabolites, whereas the ^{14}C in the acetonitrile eluant was considered to be 2,3-DCP. All samples were counted in a Packard Model 460C liquid scintillation spectrometer with sufficient counts accumulated to give <5% error with a 95% confidence interval. Quench

correction was determined using the automatic external standard method.

Statistical analysis. Data for urinary and fecal excretion of ^{14}C or ^{14}C exhaled as $^{14}CO_2$ were obtained for each rat and expressed as a fraction of the total amount excreted by each route with time after exposure. Data for all rats were pooled and expressed as the fraction remaining to be excreted as a function of time for ^{14}C in urine, feces, or CO_2. The data were fit to a nonlinear function by least-squares regression analysis using a pseudo-Gauss–Newton algorithm (BMDP, University of California, 1979). Half-times for excretion of ^{14}C were determined from the equation $t1/2 = 0.693/k_{el}$, where k_{el} (hr^{-1}) is the apparent rate constant for elimination obtained from regression analysis.

Concentrations of ^{14}C in tissues (nmol/g) from individual rats were obtained. Data for all rats were pooled and expressed as concentration of ^{14}C in each tissue as a function of time after end of exposure. The data were fit to a nonlinear function by least-squares regression analysis using a pseudo-Gauss–Newton algorithm (BMDP), and the half-times were calculated as described above.

RESULTS

2,3-[^{14}C]DCP equivalents were excreted in urine, feces, and expired air of rats exposed for 6 hr to 2,3-[^{14}C]DCP. Table 1 shows the distribution of ^{14}C in urine, feces, and expired air from rats 65 hr after the 6-hr exposure. Elimination of ^{14}C in urine was the major route of excretion with about 55% (3600 nmol) of the total absorbed 2,3-[^{14}C]DCP excreted in urine. Considerably smaller quantities of ^{14}C were excreted in the feces, with about 17% (1100 nmol) of the total absorbed 2,3-[^{14}C]DCP excreted by this route. Approximately 1 and 3% of the total absorbed 2,3-[^{14}C]DCP were exhaled as either 2,3-[^{14}C]DCP or $^{14}CO_2$, respectively. The remainder of the ^{14}C was in the carcass.

TABLE 1

DISTRIBUTION OF ^{14}C IN URINE, FECES, AND EXPIRED AIR OF RATS 65 hr AFTER EXPOSURE OF RATS FOR 6 hr TO 250 nmol 2,3-[^{14}C]DCP/liter AIR

	nmol 2,3-[^{14}C]DCP equivalents	% of absorbed 2,3-[^{14}C]DCP[a]
Urine	3585	54.6
Feces	1104	16.8
Expired air		
CO_2	211	3.2
2,3-DCP	76	1.2

[a] Total amount of 2,3-[^{14}C]DCP absorbed was determined using estimates of the minute volume (200 ml/min) and pulmonary absorption (38%) measured in a previous study (Dutcher *et al.*, 1984). Values presented represent data from four rats.

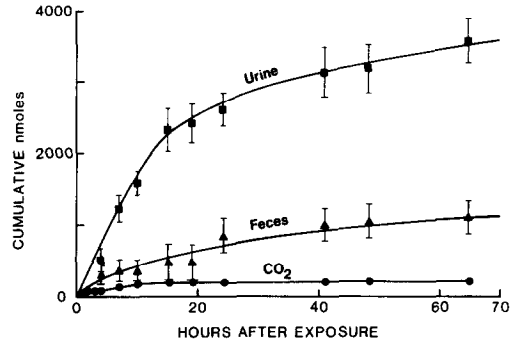

FIG. 1. Cumulative excretion in rats of ^{14}C in urine, feces, and CO_2 after a 6-hr exposure to 250 nmol 2,3-[^{14}C]DCP/liter air. Immediately after exposure, rats were placed in metabolism cages for collection of excreta as described under Methods. Each point represents $\bar{x} \pm$ SE of four rats.

Cumulative excretion of ^{14}C in urine, feces, and expired air (CO_2) is shown in Fig. 1. Approximately 75% of the ^{14}C excreted in urine and feces was eliminated within 24 hr. The rates of excretion of ^{14}C in urine, feces, and expired air were determined, and the data obtained from all rats were analyzed separately and fit to a nonlinear function. The data for urine and feces were each fitted to a one-component negative exponential function

$$F(t) = e^{-kt},$$

where $F(t)$ is the fraction remaining to be excreted at time t (hr). The data for expired air (CO_2) were fitted to a two-component negative exponential function

$$F(t) = Ae^{-k_a t} + Be^{-k_b t},$$

where $F(t)$ is the fraction remaining to be excreted at time t (hr), and A and B are the fractions eliminated with an apparent rate constant of k_a and k_b, respectively. Half-times for excretion of ^{14}C in urine, feces, and

expired air are shown in Table 2. Half-times for elimination of ^{14}C in both urine and feces were approximately 10 and 13 hr, respectively. CO_2 excretion exhibited a biphasic elimination pattern, with about 87% of the $^{14}CO_2$ being excreted with a half-time of about 3 hr and 13% excreted with a half-time of 20 hr.

Concentrations of ^{14}C in blood increased during 240 min of exposure of rats to 2,3-[^{14}C]DCP, after which no further increases in the blood concentration of ^{14}C were seen (Fig. 2). Peak concentrations of ^{14}C in the blood reached about 8 nmol/ml blood. Elimination of ^{14}C in blood after the 6-hr exposure followed a two-component negative exponential (Fig. 2). Approximately 50% of the ^{14}C in blood was eliminated with a half-time of 2.4 hr, with the remaining ^{14}C eliminated with a half-time of 113 hr (Table 3).

TABLE 2

HALF-TIMES FOR ELIMINATION OF ^{14}C FROM URINE, FECES, AND EXPIRED AIR (CO_2) FROM RATS EXPOSED FOR 6 hr TO 250 nmol 2,3-[^{14}C]DCP/liter AIR[a]

	k_{el} (hr^{-1} ± SD)[b]	Half-time (hr ± SE)
Urine	0.071 ± 0.002	9.8 ± 0.05
Feces	0.054 ± 0.003	12.9 ± 0.14
Expired air		
CO_2	0.202 ± 0.002	3.4 ± 0.10
	0.035 ± 0.007	19.7 ± 0.21

[a] Each point represents $\bar{x}$ ± SE of four rats.

[b] Data for urinary and fecal excretion of ^{14}C or ^{14}C exhaled as $^{14}CO_2$ exhaled was obtained for each rat and expressed as a fraction of the total amount excreted with time after exposure. The data were fit to a nonlinear function by least-squares regression analysis. Half-times for excretion of ^{14}C were determined from the equation $t_{\frac{1}{2}} = 0.693/k_{el}$, where k_{el} is the apparent rate constant for elimination obtained from regression analysis. The equations for the best fits were

$$\text{Urine } F(t) = e^{-kt}$$

$$\text{Feces } F(t) = e^{-kt}$$

$$CO_2\ F(t) = 0.87\ e^{-kt} + 0.13\ e^{-kt}$$

where $F(t)$ is the fraction remaining to be excreted at time t(hr).

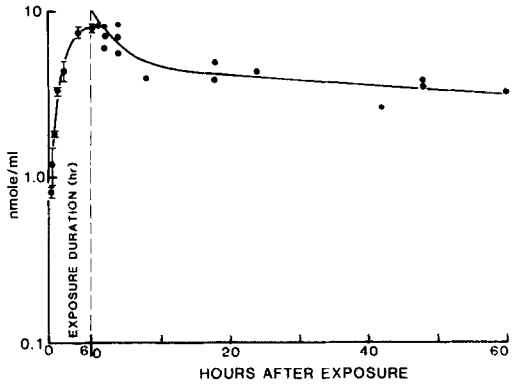

FIG. 2. Appearance and disappearance of ^{14}C in blood from rats exposed to 250 nmol 2,3-[^{14}C]DCP/liter air for various times up to 6 hr. Rats were exposed to vapors of 2,3-DCP for the designated times (see Methods), after which they were immediately euthanized with 1 ml of T-61. Blood was withdrawn from each rat by cardiac puncture. Each point represents $\bar{x}$ ± SE of three rats. At various times after exposure, groups of rats were euthanized with 1 ml of T-61 and blood was withdrawn by cardiac puncture. Individual data points were plotted and the data fit to a two-component negative exponential. The equation for the best fit was

$$F(t) = 5.4e^{-0.284t} + 4.6e^{-0.006t}$$

where $F(t)$ is the fraction remaining to be excreted at time t (hr).

^{14}C was widely distributed in tissues following the 6-hr exposure of rats to 2,3-[^{14}C]DCP. Radioactivity in tissues immediately after exposure accounted for about 9% of the estimated total 2,3-[^{14}C]DCP absorbed. Table 4 shows the concentration of 2,3-[^{14}C]DCP equivalents in tissues from rats exposed for 6 hr to 2,3-[^{14}C]DCP. Urinary bladder (150 nmol/g), nasal turbinates (125 nmol/g), kidneys (84 nmol/g), small intestine (61 nmol/g), and liver (35 nmol/g) were tissues with the highest concentrations of ^{14}C immediately after exposure. Considerably smaller concentrations of ^{14}C were detected in other tissues (Table 4). Radioactivity in carcass (including muscle, bone, pelt, and fat) immediately after exposure was approximately 15% of the 2,3-[^{14}C]DCP absorbed (data not shown). The kidneys and liver were the only two tissues examined immediately after exposure that contained as much as between 2 and 5%, respectively, of the ab-

TABLE 3

HALF-TIMES FOR DISAPPEARANCE OF ^{14}C FROM VARIOUS TISSUES OF RATS EXPOSED FOR 6 hr TO 250 nmol 2,3-[^{14}C]DCP/liter AIR[a]

	K (hr^{-1} ± SD)[b]	Half-time (hr ± SE)
Lung	0.156 ± 0.006	4.4 ± 0.3
Liver	0.103 ± 0.003	6.7 ± 0.4
Larynx	0.063 ± 0.002	11.0 ± 0.7
Nasal turbinates	0.094 ± 0.003	7.4 ± 0.8
Small intestine	0.151 ± 0.005	4.6 ± 0.3
Testes	0.254 ± 0.005	2.7 ± 0.1
Trachea	0.087 ± 0.004	8.0 ± 0.8
Kidney	0.248 ± 0.006	2.8 ± 0.1
Blood[c]		
1st Component	0.284 ± 0.173	2.4 ± 0.3
2nd Component	0.006 ± 0.004	113.6 ± 18.7

[a] Each point represents $\bar{x}$ ± SE of 2 to 3 rats.

[b] Concentrations of ^{14}C in tissues (nmol/g) from individual rats were obtained. Data for all rats were pooled and expressed as concentration of ^{14}C in each tissue as a function of time after end of exposure. The data were fit to a nonlinear function by least-squares regression analysis. Half-times for disappearance of ^{14}C from tissues were determined from the equation $t_{\frac{1}{2}} = 0.693/k_{el}$, where k_{el} is the apparent rate constant for disappearance of ^{14}C obtained from regression analysis. The equation used to fit the data was $F(t) = Ae^{-kt} + B$, where $F(t)$ is the fraction remaining to be excreted at time t(hr), A is the fraction eliminated with an apparent rate constant of k, and B is a constant.

[c] Data from blood were fit to a two-component negative exponential function.

sorbed 2,3-DCP (Table 4). The ^{14}C in other tissues immediately after exposure typically was approximately less than 1% of the 2,3-[^{14}C]DCP absorbed (Table 4).

Data from tissues of individual rats were fit to the following equation:

$$F(t) = Ae^{-kt} + B,$$

where $F(t)$ is the fraction remaining to be excreted at time t (hr), A is the fraction eliminated with an apparent rate constant of k, and B is a constant. In most cases, nearly 80% of the initial amount of ^{14}C in tissues immediately after exposure was eliminated by 65 hr. Table 3 shows the half-times for disappearance of 2,3-[^{14}C]DCP equivalents from various tissues. Elimination half-times of ^{14}C were about 2.5 to 11 hr. In nearly all tissues examined, there appeared to be a small fraction of the total radioactivity in the tissues which was removed from the tissues with a very long half-time. The data in Table 4 indicate that by 65 hr after exposure, most of the ^{14}C in the tissues was eliminated.

Liver, lungs, kidneys, and blood contained sufficient ^{14}C for analysis of 2,3-[^{14}C]DCP metabolites at 1, 2, and 4 hr postexposure. In all cases, more than 90% of the ^{14}C in the tissues examined was 2,3-DCP metabolites within 2 hr after the end of exposure. Analysis of urine and feces samples in which there was sufficient radioactivity indicated that all the ^{14}C was contained in 2,3-DCP metabolites. No evidence of parent compound was found in any of the excreta samples analyzed. No attempt was made to identify these metabolites.

DISCUSSION

The data presented in this report indicate that 2,3-DCP is extensively metabolized and excreted by rats exposed for 6 hr to 2,3-[^{14}C]DCP vapor. The major route of elimination of inhaled 2,3-[^{14}C]DCP was urinary excretion, an observation similar to that seen in rats orally dosed with 1,3-[^{14}C]DCP (Hutson *et al.*, 1971; Dietz *et al.*, 1984b) and 2,3-[^{14}C]DCP (Dutcher *et al.*, 1984; Medinsky *et al.*, 1984). Hutson *et al.* (1971) have previously reported that metabolism of *cis*-1,3-DCP to CO_2 was a minor metabolic pathway, accounting for only 5% of the administered dose. However, metabolism of *trans*-1,3-DCP to CO_2 accounted for more than 20% of the initial dose, suggesting that the two isomers of 1,3-DCP were metabolized through different metabolic pathways (Hutson *et al.*, 1971). The results from a previous study (Medinsky *et al.*, 1984) in which rats were dosed either orally or intraperitoneally with 2,3-[^{14}C]DCP are similar to the observations reported in this study in regard to CO_2 excretion.

2,3-DCP was extensively metabolized, with more than 90% of the ^{14}C in liver, kidneys, and lungs identified as 2,3-DCP metabolites

TABLE 4

TISSUE CONCENTRATIONS (nmol/g) OF RADIOACTIVITY DERIVED FROM 2,3-[^{14}C]DCP IN RATS EXPOSED FOR 6 hr TO 250 nmol 2,3-[^{14}C]DCP/liter AIR[a]

	0 Hr	8 Hr	24 Hr	60 Hr
Urinary bladder	148 ± 46.9 (0.19)	7.2 ± 4.8 (0.02)	3.9 ± 0.0 (0.01)	2.0 ± 0.3 (0.00)
Turbinates	126 ± 35.6 (0.32)	58.4 ± 17.3 (0.15)	31.1 ± 0.0 (0.09)	25.6 ± 8.8 (0.08)
Kidneys	84.2 ± 3.3 (2.40)	25.8 ± 9.0 (0.82)	21.2 ± 0.4 (0.63)	14.1 ± 0.6 (0.44)
Small intestine	61.5 ± 9.9 (0.67)	15.6 ± 9.8 (0.23)	2.4 ± 0.0 (0.04)	1.3 ± 0.2 (0.03)
Liver	35.5 ± 1.0 (4.76)	13.8 ± 3.4 (1.97)	12.5 ± 0.9 (1.88)	6.4 ± 0.4 (0.89)
Trachea	15.6 ± 1.0 (0.03)	11.5 ± 3.7 (0.02)	8.4 ± 0.0 (0.01)	5.5 ± 0.3 (0.01)
Larynx	11.9 ± 0.4 (0.01)	6.2 ± 3.7 (0.01)	4.4 ± 0.0 (0.00)	2.3 ± 0.2 (0.00)
Adrenals	11.1 ± 1.2 (0.01)	2.9 ± 0.8 (0.00)	2.4 ± 0.0 (0.01)	2.0 ± 0.3 (0.00)
Large intestine	10.8 ± 0.8 (0.05)	7.5 ± 3.7 (0.11)	3.8 ± 0.7 (0.06)	2.0 ± 0.2 (0.03)
Testes	10.7 ± 0.8 (0.56)	2.4 ± 0.9 (0.14)	1.3 ± 0.0 (0.08)	1.2 ± 0.1 (0.06)
Lung	10.1 ± 0.4 (0.13)	6.7 ± 2.0 (0.12)	4.1 ± 0.3 (0.10)	3.9 ± 0.2 (0.07)
Thymus	10.0 ± 0.5 (0.04)	3.2 ± 2.8 (0.02)	3.6 ± 0.0 (0.02)	1.9 ± 0.1 (0.01)
Thyroid	8.9 ± 1.2 (0.00)	2.9 ± 0.9 (0.00)	2.4 ± 0.0 (0.01)	2.3 ± 0.7 (0.00)
Stomach	7.9 ± 0.5 (0.16)	2.8 ± 1.0 (0.06)	1.8 ± 0.1 (0.03)	1.4 ± 0.1 (0.03)
Spleen	7.8 ± 0.6 (0.06)	3.0 ± 0.9 (0.02)	2.5 ± 0.1 (0.05)	1.9 ± 0.1 (0.02)
Brain	7.2 ± 0.5 (0.15)	2.1 ± 0.8 (0.05)	1.1 ± 0.0 (0.01)	1.1 ± 0.1 (0.02)
Heart	5.6 ± 1.4 (0.08)	1.9 ± 0.4 (0.03)	1.7 ± 0.2 (0.03)	1.7 ± 0.2 (0.03)
Fat	8.5 ± 1.0 (3.8)	1.1 ± 0.5 (0.4)	1.3 ± 0.5 (0.4)	0.8 ± 0.2 (0.2)

[a] Each value represents $\bar{x}$ ± SE for 2 to 3 rats. Values in parentheses represent the percentage of estimated adsorbed 2,3-DCP recovered in tissues.

within 2 hr postexposure. Studies by Climie *et al.* (1979) indicate that glutathione plays a major role in the biotransformation of 1,3-DCP. Climie *et al.* (1979) have shown that both *cis*- and *trans*-1,3-DCP are metabolized primarily to the mercapturic acid, *N*-acetyl-(3-chloroprop-2-enyl)cysteine. However, the glutathione-dependent conjugation of *cis*-1,3-DCP was more than 4 to 5 times the rate of conjugation of *trans*-1,3-DCP. Dietz *et al.* (1984a) also demonstrated that a mixture of *cis, trans*-1,3-DCP was metabolized to the mercapturic acid, *N*-acetyl-(3-chloroprop-2-enyl)cysteine. Dietz *et al.* (1984a) have shown that glutathione depletion in rats was correlated with increased covalent binding of 1,3-DCP to forestomach.

Although no attempt was made in these studies to identify the metabolites present in excreta or tissues, the observation that the excretion patterns of 2,3-DCP are similar to *cis*-1,3-DCP suggest that 2,3-DCP may be metabolized to mercapturic acids in a manner similar to that seen for *cis*-1,3-DCP. Presumably reaction of the 2,3-DCP would involve conjugation with glutathione at the carbon-3 to form the mercapturic acid of 2,3-DCP. Alternatively, glutathione could react with a 1,2-epoxide of 2,3-DCP. However, theoretical considerations reveal that six different reactive intermediates of 2,3-DCP are possible, each with the capability to alkylate DNA (Eder *et al.*, 1982).

^{14}C was widely distributed in the body, with all tissues examined containing ^{14}C. Data presented in this study indicate that ^{14}C is cleared rapidly from tissues. These observations are consistent with those reported for 2,3-DCP (Dutcher *et al.*, 1984; Medinsky *et al.*, 1984) and 1,3-DCP (Hutson *et al.*, 1971).

The data from the present study indicate that the kidneys and nasal turbinates are the tissues with the highest concentrations of ^{14}C 65 hr after exposure to 2,3-[^{14}C]DCP. Nasal tissue contains cytochrome *P*-450-dependent monoxygenases capable of metabolizing a variety of xenobiotics (Dahl *et al.*, 1982; Hadley and Dahl, 1982; Bond, 1983). Metabolism of 2,3-DCP to reactive intermediates by the nasal tissue or kidneys could play an

important role in the metabolic fate of inhaled 2,3-DCP. Whether 2,3-DCP has a toxic effect on these tissues remains to be determined.

In summary, urinary excretion was the major route of elimination of ^{14}C from rats exposed to vapors of 2,3-[^{14}C]DCP. ^{14}C was widely distributed in tissues, over 90% of which appeared to be 2,3-DCP metabolites. The data indicate that after inhalation, 2,3-DCP is metabolized in tissues and excreted.

ACKNOWLEDGMENTS

The present research was conducted under US Department of Energy (DOE) contract number DE-AC04-76EV01013 through an interagency agreement (222-Y02-ES-0092) with the National Institute of Environmental Health Sciences as part of the National Toxicology Program. The facilities used for this research were fully accredited by the American Association for Accreditation of Laboratory Animal Care. The authors gratefully acknowledge the excellent technical assistance of Ms. E. Cahill, Mr. M. Malone, Ms. F. Straus, Ms. M. Steuver, and Ms. A. Swanzy and the useful discussions with a number of our colleagues at the Institute, in particular, Dr. R. O. McClellan.

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