Metabolism and Tissue Distribution of (1, 4, 5, 8-^{14}C)—1, 2-Dichloronaphthalene in Rats

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Polychlorinated naphthalenes (PCN) are widely used industrial compounds with properties similar to the polychlorobiphenyls (HARDIE, 1964; GOERLITZ and LAW, 1972). PCN are known as causative agents of X-disease of cattle (SIKES and BRIDGE, 1952) and chloracne in humans (KLEINFELD et al., 1972). Recently PCN were detected in polychlorobiphenyl-contaminated environmental samples (CRUMP-WEISNER. et al., 1973; GOERLITZ and LAW, 1974). Although some reports described the identification of metabolites derived from isomeric chloronaphthalenes, little information is available on their metabolic fate and pharmacokinetic behavior (RUZO et al., 1975 and 1976). The present studies were carried out as part of a general program on PCN and were designed to determine the metabolism, absorption, distribution and excretion of 1,2-dichloronaphthalene, a major component of Halowax 1031 and 1000 (BELAND and GEER, 1973).

Materials and Methods

Synthesis of (1,4,5,8-^{14}C)-1,2-Dichloronaphthalene (1,4,5,8-^{14}C)-2-Naphthol, 17 mg, specific activity of 4.2 mCi/mmol (Amersham Searle, Chicago) was diluted with unlabeled 2-naphthol (973 mg), and dissolved in 5% aqueous sodium hydroxide (6 ml) at 5° C. Sodium hypochlorite (4.1 ml, 0.127 gm/ml) was added to this solution dropwise with stirring, and the mixture was stirred for 15 min. after the completion of addition. The reaction mixture was acidified with 10% HCl, extracted with chloroform (3 X 10 ml), and combined chloroform extracts evaporated to give (1,4,5,8-^{14}C)-1-chloro-2-naphthol as a pink solid (1142 mg). The specific activity was 0.40 μCi/mg.

A mixture of (1,4,5,8-^{14}C)-1-chloro-2-naphthol (1115 mg), phosphorus pentachloride (1370 mg) and phosphorus oxychloride (0.18 ml) was heated in an oil bath at 170° for 12 hours. The mixture was poured onto crushed ice (100 g), extracted with chloroform (3 X 50 ml) and the combined extracts were evaporated to give (1,4,5,8-^{14}C)-1,2-dichloronaphthalene (DCN) as a yellow liquid (bp. 150-154°/3mm) which solidified upon standing at room temperature. Mp 65-66°, lit. mp 68-69° (HEILBRON, 1965). The specific activity was 0.365 μCi/mg. Radiochemical purity was found to be greater than 99%.

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This compound was diluted with unlabeled 1,2-dichloronaphthalene to give 0.05 μCi/mg and used for all experiments.

All scintillation countings were performed on a Mark III Liquid Scintillation System (Amersham Searle, Chicago). Quench was corrected by use of an external standard. Detection of metabolites after thin layer chromatography was carried out using an Actigraph scanner (Amersham Searle, Chicago).

Absorption Studies

Male Wistar Rats (250-350 g) were used for all experiments. The jugular vein of four rats was cannulated with PE-50 tubing. After a week of recuperation the animals were given a single oral dose of DCN, 20 μCi/kg (400 mg/kg) in corn oil. Serial blood samples (0.2 ml) were withdrawn from the jugular vein at 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hour intervals for the analysis of radioactivity. After the 48 hour sampling the four animals were killed by ether asphyxiation, organs were excised, weighed and analyzed for the 48 hour tissue distribution study.

Distribution and Excretion

Ten rats were intubated with a single oral dose of DCN (20 μCi/kg), placed in metabolic cages, with food and water ad lib. Five animals were sacrificed for 24 hour tissue distribution studies, while the remaining five rats were kept for one week and then killed to determine the residual radioactivity in tissues.

Biliary Excretion

Two rats were anesthetized with fluothane and the common bile duct was cannulated with PE-10 tubing. DCN (10 μCi/kg in corn oil) was administered via the genital vein. Serial bile samples were collected hourly for eight hours, then at 24, 48 and 72 hour intervals. An aliquot of bile (100 μl) was measured for radioactivity.

Preparation of Scintillation Counting Samples

Urine (50 μl) and bile (100 μl) were dissolved in Bray's reagent (15 ml, New England Nuclear) and were counted directly.

Powdered dried tissue (100 mg) and feces (50 mg) were rehydrated with water (0.2 ml), digested with Soluene-350 (2 ml, Packard) at 50° C overnight and counted in Aquasol (10 ml, Packard).

The blood samples (0.2 ml) were dissolved in a mixture of 2-propanol/Soluene-350) (1 ml, Packard), decolorized with 30% hydrogen peroxide (0.5 ml) and incubated at 50° for 15 min. The radioactivity

was determined in 15 ml of 0.5 N HCl/Instagel (1:9, Packard).

Enzyme Hydrolysis

A mixture of urine (1 ml) and β-glucuronidase (Glucurase, 1 ml, 5000 Sigma unit) was incubated at 37° for 24 hours. Fifty microliters of the incubated mixture was applied to the thin layer chromatography plate (Eastman Kodak 6060), developed in a solvent system of benzene: dioxane: acetic acid (90:25: 8) and scanned for radioactivity using Actigraph scanner.

The band containing radioactive material was removed from the plate and extracted with chloroform. The solid material that obtained after evaporating the solvent was analyzed by GC-MS (Varian Mat 311 A) with a 6' x 1/8" id glass column packed with 3% OV-17 on 80-100 mesh Chromosorb W-HP.

Dry feces (2 g) were extracted with ethanol (3 X 20 ml) and the combined extracts were evaporated to dryness. The residue was treated with Glucurase in a manner similar to that described for the urine.

Results and Discussion

Absorption Studies

The radioactivity content of blood at specified time intervals after the oral administration of DCN is shown in Table 1.

TABLE 1

Radioactivity (DPM) of the blood after a single oral administration of DCN, 20 uCi/kg. The S.D. are within 40% of the means.

Time (hour)	Radioactivity (DPM)*/0.2 ml
1	15278
2	14399
3	10087
4	13088
5	13237
6	12048
7	11430
8	9782
24	5492
48	2870

*The figures represent the average value of three rats.

DCN is rapidly absorbed with the highest level of radioactivity at 1 hr and gradually declines over the first 8 hours. After 48 hours the levels of radioactivity in blood are approximately 30 and 15% respectively of the level observed after 1 hour. These levels reflect the combined effects of absorption, metabolism and the equilibrium between blood and tissues.

Tissue Distribution

The results for the tissue distribution studies are shown in Table 2.

TABLE 2

Tissue distribution* of radioactivity in rats 24 hours (A), 48 hours (B) and 7 days (C) after a single oral dose of DCN in DPM/mg dried tissue and % of total dose given to the test animals. The S.D. are within 40% of the means.

	A		B		C	
	DPM/mg	%	DPM/mg	%	DPM/mg	%
Adipose	8.1	0.1	11.7	0.15	3.26	0.04
Lung	14.1	0.04	14.3	0.03	-	-
Liver	21.3	0.7	34.3	0.07	-	-
Bladder	30.6	0.01	42.4	0.01	-	-
Kidney	39.6	0.18	46.5	0.15	-	-
Intestine	87.0	0.45	251	3.6	-	-
Skin	4.7	0.07	5.7	0.08	0.73	0.01
GI-content	1124	18.3	1963	17.9	7.6	0.04
Fecal excretion		18.9		30.8		42.0
Urine		26.4		32.6		35.2

*The average value of four or more animals.

The highest levels of radioactivity (DPM/mg) were found in liver, kidneys, intestine, bladder and adipose tissue. When radioactivity is expressed in terms of percent of the total dose that test animal received, only liver and intestine are most important in distribution because these organs are comparatively large. No significant differences in radioactivity levels of tissue were noted between 24 and 48 hour samples. After seven days virtually no radioactivity was detected in tissues except for skin and adipose tissue. The slow removal of the radioactivity from skin and adipose tissue was probably due to the lipophilic nature of DCN.

Excretion

Analysis of the urine and feces showed that most of the radioactivity was eliminated via these routes (Figure 1). The rate

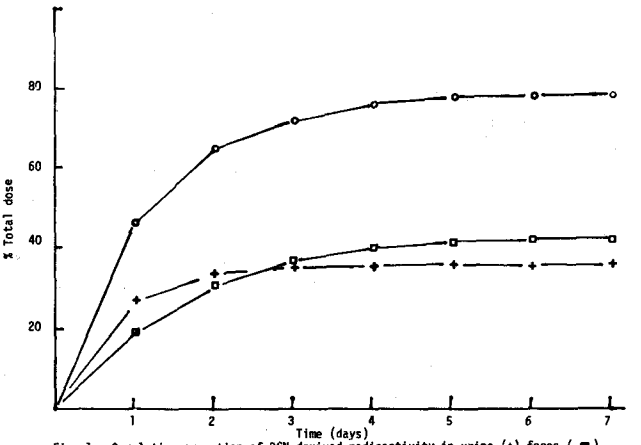

Fig. 1. Cumulative excretion of DCN-derived radioactivity in urine,(+) feces (◘) and the total excretion (O) after a single oral administration of the test compound at 20 μCi/kg. Data are the means from five rats and S.D. are within 40% of the means.

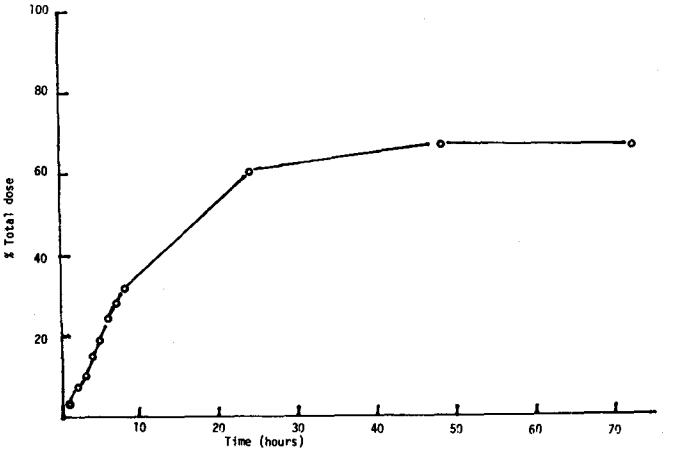

Fig. 2. Cumulative excretion of radioactivity from bile after an I.V. administration of 10 μCi/kg to two biliary cannulated rats. The rate of excretion in the two rats is almost identical.

of excretion was rapid and 64% of the dose was removed in the excreta within two days. Twenty six percent of the total dose was excreted in the urine in the first day, 33% in two days, and a total of 35% in seven days. The first day feces accounted for 19% of the total dose, while 31% was removed in two days. The amount of radioactivity excreted via the feces in seven days amounted to 42% of the original dose. Collection of urine and feces was discontinued after seven days because radioactivity could not be detected.

To ascertain if there was biliary excretion of DCN or its derived materials, serial bile samples were analyzed for radioactivity. It was found that more than 62% of the radioactivity was removed in bile within 24 hours while excretion was insignificant thereafter. (Figure 2). It was observed that only 42% of the total dose was excreted in feces while 65% entered into the gastro-intestinal tract via bile. This suggests DCN or its metabolites are reabsorbed from the intestine and excreted into the bile.

Analysis of the fecal extract by TLC showed that the radioactivity was due to unchanged DCN. GC-MS of this compound gave a molecular ion at m/e 196 and 198 (M/M + 1 = 3/2) characteristic of a dichloronaphthalene. No DCN or free chloronaphthol could be detected in the urine. The metabolite associated with urine is the glucuronide of a dihydrodiol of DCN since it was isolated after hydrolysis with β-glucuronidase. The hydrolysate has a molecular ion of m/e 230, 232 (M/M + 1 = 3/2), and a fragment at m/e 212 ($M-H_2O$). The accurate mass of the molecular ion of 229.9899 (theoretical value for $C_{10}H_8O_2Cl_2$ = 229,9901) further supports this assignment. This finding is consistent with the reported metabolic pathway of aromatic compounds in which arene oxide is the proposed intermediate (DALY et al., 1972). Our results also point out for the first time a dihydrodiol formation in chlorinated naphthalenes and provide an alternate metabolic pathway for these compounds (RUZO et al. 1975 and 1976, CHU et al. 1976). The position of hydroxylation is not known at the present but determination of the precise structure of the hydroxylated metabolite is in progress and will be published elsewhere.

In summary DCN was found rapidly absorbed in the GI tract, metabolized to dihydrodiol. Forty two per cent of the DCN was excreted unchanged via feces, while 35% appeared in urine as the glucuronide of a dihydrodiol.

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