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Distribution and Excretion of Mono*iso*propylnaphthalene in Rats

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Isopropylnaphthalenes have recently been used in duplicating papers and heat-transfer media as a substitute for polychlorinated biphenyls, which have been recognized as dangerous environmental contaminants (PEAKALL and LINEER 1970; EDWARDS 1971). In view of the increase in the use of isopropylnaphthalenes, it is desirable to study the toxicity and biological fate of isopropylnaphthalenes, in which their release into the environment appears inevitable, in animals. Recently, we studied the absorption, tissue distribution, and excretion of unchanged 2-isopropylnaphthalene (2-IPN) in rats after single and continuous oral administration of 2-IPN (KOJIMA and MARUYAMA 1979), and also reported the identification of urinary metabolites of 2-IPN in rats (KOJIMA et al. 1980).

In the present study, in order to examine in detail the tissue distribution and urinary and fecal excretions of both unchanged compound and metabolite of monoisopropylnaphthalene (MIPN), the distribution and excretion of monoisopropyl[^{14}C]naphthalene (MIPN-^{14}C) were studied in male rats after oral administration.

MATERIALS AND METHODS

Chemicals and equipments: MIPN-^{14}C (sp. activity, 5.65 μCi/mg), which consisted of 2-isopropyl[^{14}C]naphthalene (60%) and 1-isopropyl[^{14}C]naphthalene (40%), 2-IPN (bp 268°C), and 1-isopropylnaphthalene (1-IPN, bp 268°C) were a gift from Kureha Chemical Co. (Tokyo). PCS liquid scintillation cocktail was purchased from Amersham Corp. (Arlington Heights, Ill.). Carbo-Sorb carbon dioxide absorber and Permafluor V liquid scintillation cocktail were purchased from Packard Instrument Co. Inc. (Downers Grove, Ill.). All other chemicals were of reagent grade. An Aloka LSC-502 liquid scintillation counter, a Packard Tri-Carb sample oxidizer (model 306), and a Shimadzu gas chromatograph (model GC-3BF) were utilized.

Oral administration experiment: 1) MIPN-^{14}C: Male Wistar rats weighing 190-210 g were used. Rats were administered a single dose of MIPN-^{14}C (6.25 μCi/kg) plus

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non-labeled MIPN (60% 2-IPN and 40% 1-IPN)(100 mg/kg) in an olive oil (0.4 ml) by a stomach tube. The animals were housed in individual metabolic cages with diet and water ad libitum. The feces and urine were collected separately 24, 48, 72 and 96 hr after administration of MIPN-^{14}C. The animals were sacrificed by decapitation at 2, 4, 6, 12 and 24 hr after administration. The various tissues were collected and stored in a freezer until they were applied to analysis.

2) 1-IPN: 1-IPN (100 mg/kg) was given as an olive oil solution to rats by a stomach tube. The feces, urine, and various tissues were collected 2, 6, 12 and 24 hr after administration as mentioned above.

In situ rat biliary excretion experiment: Male Wistar rats, weighing 170-220 g, were anesthetized with ethyl ether and the bile duct was cannulated with polyethylene tubing (PE-10) as described previously (KOJIMA and MARUYAMA 1979). After oral administration of MIPN-^{14}C as an olive oil solution, the rat was housed in a Bollman cage with diet and water ad libitum, and the bile was collected 2, 4, 6, 8, 24 and 48 hr after administration.

Measurement of MIPN-^{14}C radioactivity in samples: 1) Tissues: Each tissue (100 mg) was decolorized by warming at 45°C for 3-5 hr in a vial with 0.3 ml of 30% H_2O_2 and then dissolved by adding 1.2 ml of NCS solubilizer and warming at 45°C for 5-6 hr. The resulting solution was neutralized by adding 0.05 ml of glacial acetic acid. A portion (10 ml) of PCS liquid scintillation cocktail was added to each vial, and the radioactivity was determined using a liquid scintillation counter.

2) Urine and bile: Total radioactivity in the urine or bile does not differentiate between unconjugated and conjugated metabolites of MIPN-^{14}C. The relative amounts of unconjugated and conjugated metabolites were determined for the urine and bile. One milliliter of the urine or bile was adjusted to pH 1.0-2.0 with N HCl and extracted twice with 1-ml portions of chloroform. The chloroform layer contained the unconjugated metabolites, which were produced by the hydroxylation and/or carboxylation of the isopropyl chain of MIPN, and the aqueous layer the conjugated metabolites such as glucuronides of metabolites described above. An aliquot (0.1 ml) of each chloroform and aqueous layer was placed in each scintillation vial containing 10 ml of PCS scintillation mixture and the radioactivity was determined as mentioned above. The radioactivities in the chloroform and aqueous layer were designated as those for the unconjugated and conjugated metabolites, respectively.

3) Feces: The feces were dried and ground into a powder with a mortar and pestle. An aliquot (100 mg) of each collection was oxidized by a Tri-Carb sample oxi-

dizer and $^{14}CO_2$ was trapped into Carbo-Sorb carbon dioxide absorber. Permafluor V scintillation cocktail was added to the trapped $^{14}CO_2$ and counted in a liquid scintillation counter.

These procedures for tissues, urine, bile and feces consistently gave the recoveries of 80-85% of the radioactivity.

Determination of unchanged 1-IPN in various tissues, urine and feces: The determination of unchanged 1-IPN in various tissues, urine and feces in rats was conducted according to the method of 2-IPN reported previously (KOJIMA and MARUYAMA 1979). Whole blood, urine, and various tissues and feces, which were each homogenized with an equal amount of anhydrous Na_2SO_4 in a mortar and pestle, were extracted with n-hexane. The n-hexane extracts were evaporated to dryness in vacuo. The residue was dissolved in an adequate volume of n-hexane containing 0.005% diphenyl (internal standard) and then subjected to GLC. Conditions for GLC were: column, 2.1 m x 3 mm glass tube containing 60-80 mesh Shimalite W coated with 10% Apiezon-L; column temperature, 190°C; and flow rate of carrier gas (N_2), 48 ml/min. These procedures for these specimens gave the recoveries of 92-98% of 2-IPN and 1-IPN.

TLC of metabolites of MIPN: The identification of metabolites of MIPN was conducted according to the chromatographic method reported previously (KOJIMA et al. 1980). Wakogel plates (Wako Pure Chemical Industries, Osaka) were used. Solvent systems used were (1) chloroform/n-hexane/acetic acid (10:2:1, v/v) and (2) n-propanol/28% ammonia water (7:3, v/v). Spots were detected with iodine vapor for the unconjugated metabolites and naphthoresorcinol reagent for the glucuronides.

RESULTS AND DISCUSSION

Since MIPN-^{14}C used in this study consisted of two components, 2-isopropyl[^{14}C]naphthalene and 1-isopropyl[^{14}C]naphthalene, the tissue distribution of unchanged compound after oral administration of 1-IPN (100 mg/kg) was compared with that of unchanged 2-IPN, which was reported previously from our laboratory (KOJIMA and MARUYAMA 1979). As shown in Figure 1, the tissue concentration pattern of unchanged 1-IPN was almost the same as that of unchanged 2-IPN. The distribution of radioactivity in various tissues in rats administered orally MIPN-^{14}C was shown in Figure 1. The data for the blood concentrations indicated much slower rate of decline of radioactivity than did each unchanged compound after administration of 1-IPN and 2-IPN, resulting in much higher blood concentration of radioactivity. These results show that the blood contains mostly the

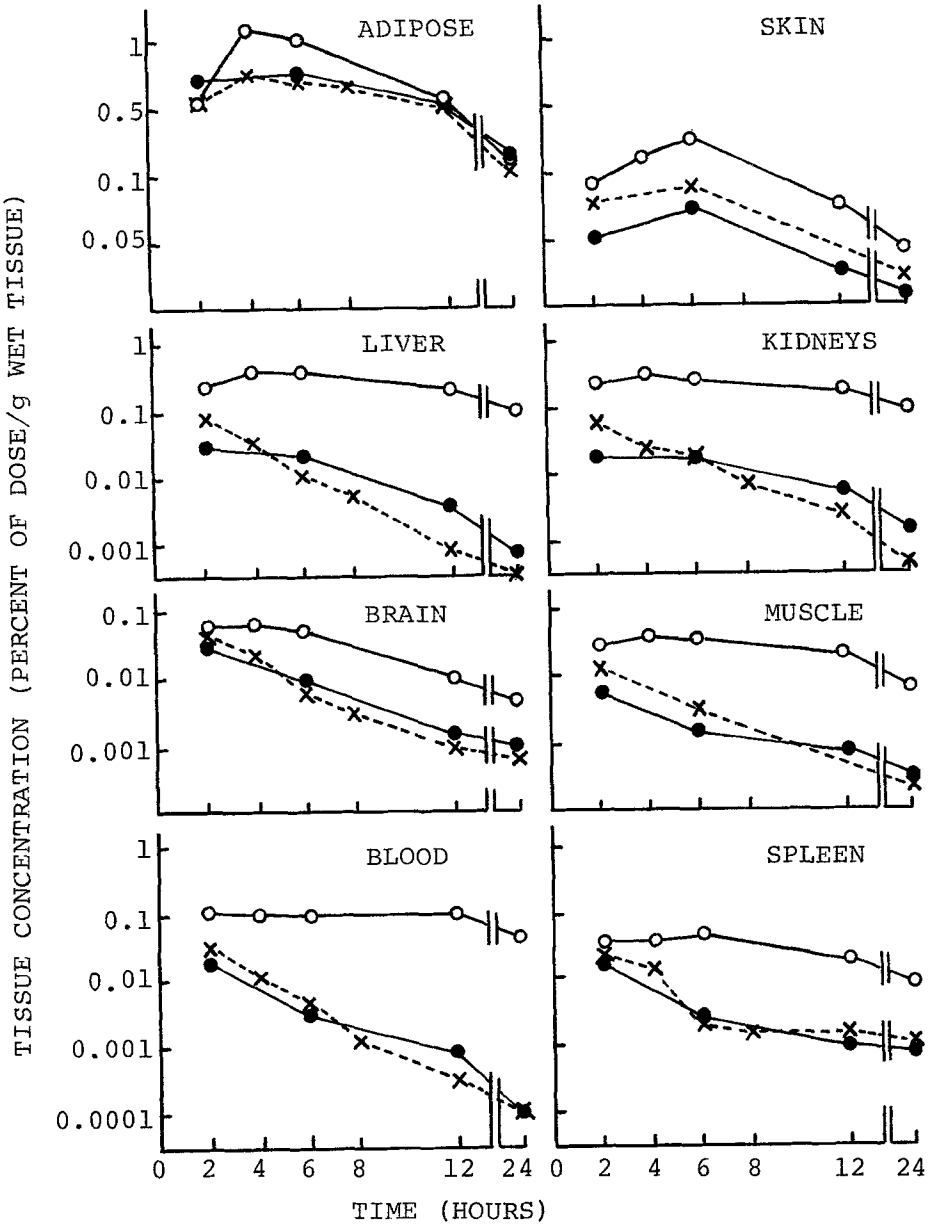

Figure 1. Tissue concentrations of radioactivity (o), unchanged 1-IPN (●) and unchanged 2-IPN (×) following single oral administration. Data for 2-IPN were cited from the previous report (KOJIMA and MARUYAMA 1979). Each value represents the mean for 3 animals.

metabolites of MIPN-^{14}C and that the disappearance of the metabolites is considerably slow. As can be seen from the concentrations of radioactivity and unchanged 1-IPN or 2-IPN in Figure 1, the relative amounts of unchanged compound and metabolite differ from tissue to tissue. Within 24 hr after administration, the liver, kidneys, muscle, and spleen contained predominantly the metabolites, whereas the adipose tissue, skin, and brain contained appreciable amounts of unchanged compound.

The tissue to blood distribution ratios for both unchanged compound and metabolite were estimated for each tissue by calculating the ratios of the respective tissue concentration to blood concentration (Table 1). The distribution ratios were higher for the unchanged compound than for the metabolite for each compound, indicating that the unchanged compounds concentrate more than the metabolites in each tissue. The distribution ratios of each unchanged compound were greater in the adipose tissue and skin than in other tissues. In addition, the distribution ratios of the metabolites in the liver and kidneys were higher than those in other tissues. These results suggest that the liver is a primary site of metabolism of MIPN and that most of its metabolites are excreted through the kidneys.

TABLE 1

Tissue to blood distribution ratios[a]

Tissue	Unchanged		Metabolite	
	1-IPN	2-IPN	1-IPN	2-IPN
Blood	1	1	1	1
Liver	4.81	2.85	2.40	2.34
Kidneys	6.74	5.48	1.98	1.90
Spleen	2.93	4.90	0.24	0.23
Brain	4.83	3.45	0.20	0.18
Muscle	1.18	1.47	0.23	0.19
Skin	39.40	58.87	0.40	0.46
Adipose	512	496	1.22	1.58

a. Experimental values of unchanged and metabolite concentrations in tissues and in blood were available at three or four time points.

In order to examine the routes of excretion of MIPN-^{14}C, we determined the amounts of radioactivity excreted in the urine and feces after oral administration of the compound (Figure 2). The major route of excretion of radioactivity derived from MIPN-^{14}C was via

the urine. Approximately 78% of the total dose was excreted in the urine during 96 hr after the administration and most of the excretion in the urine occurred during the first 24 hr. A relatively small percent of the total dose (about 14%) was excreted in the feces. These results indicated that the route of excretion of MIPN was remarkably different from that of diisopropylnaphthalene (IWAHARA 1974), in which the excretion of radioactivity in the urine and feces in rats administered ^{3}H-diisopropylnaphthalene was approximately 26% and 71% of the total dose, respectively.

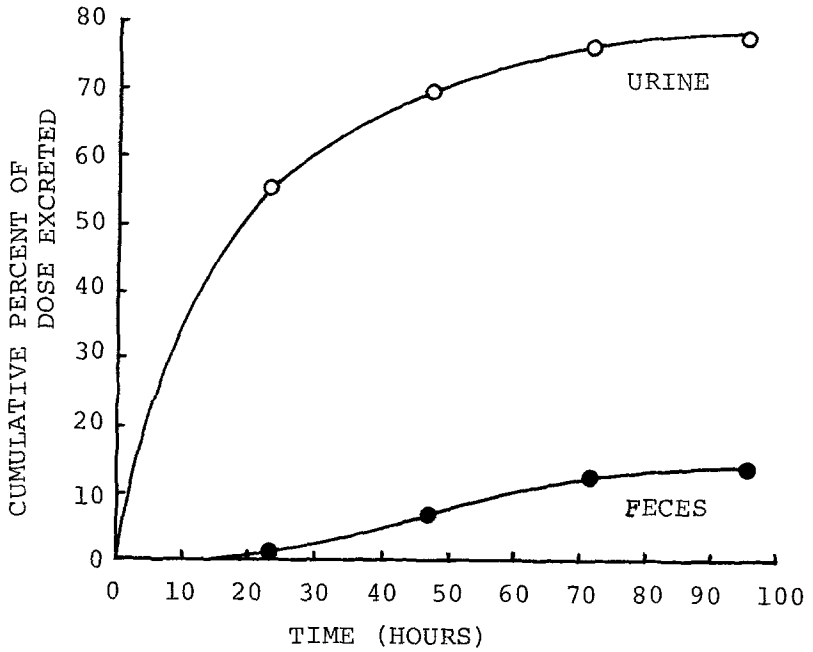

Figure 2. Cumulative excretion of radioactivity in urine and feces after oral administration of MIPN-^{14}C. Each value represents the mean for 2 animals.

In addition, in order to investigate the participation of biliary excretion in the fecal excretion of MIPN-^{14}C, the biliary excretion of radioactivity in rats administered orally MIPN-^{14}C was examined. As shown in Figure 3, the excretion of radioactivity in the bile was rapid, resulting in approximately 60% of the total dose during the first 24 hr. Judging from the finding mentioned above that the fecal excretion was the minor route of excretion of radioactivity derived from MIPN-^{14}C, it is implied that enterohepatic circulation plays an important role in the reabsorption of the metabolites excreted in the intestine via the bile.

Furthermore, the amounts of both conjugated and unconjugated metabolites of MIPN-^{14}C in the urine and bile after oral administration of MIPN-^{14}C were determined. As shown in Table 2, in either urine or bile, a large portion of the excreted radioactivity was present

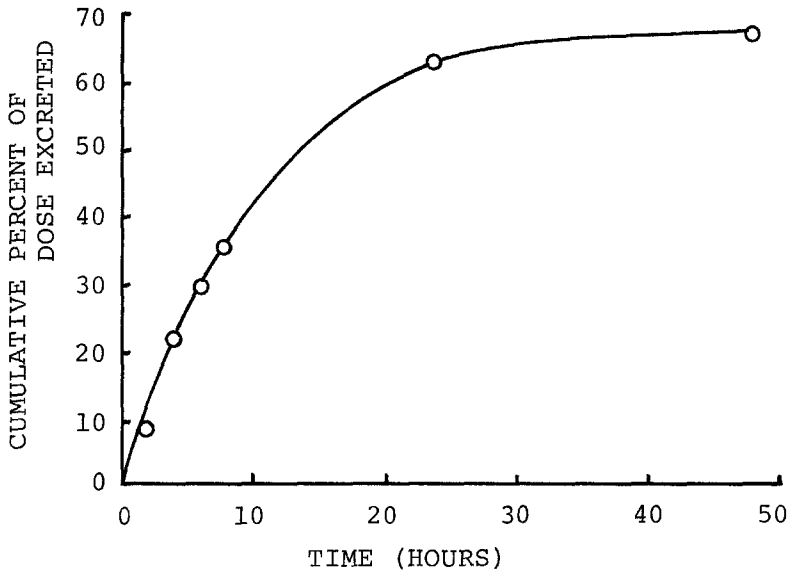

Figure 3. Cumulative excretion of radioactivity in bile after oral administration of MIPN-^{14}C. Each value represents the mean for 3 animals.

TABLE 2

Metabolites of MIPN-^{14}C in urine and bile after oral administration of the compound

Metabolite	% of total radioactivity[a]	
	Urine[b]	Bile[c]
Unconjugates	61.67 ± 6.21	74.67 ± 0.73
Conjugates	38.33 ± 6.20	25.33 ± 0.75

a. Mean ± standard deviation for 3 animals.
b. Urine was collected for 48 hr after the administration.
c. Bile was collected for 48 hr after the administration.

as the unconjugated metabolites. These results suggest that most of the unconjugated metabolites excreted in the bile undergo rapidly enterohepatic circulation and are ultimately excreted in the urine.

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