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Accumulation and Excretion of *Iso*propylchlorobiphenyls in Mouse and Fish

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Many papers are available on the metabolic conversion of polychlorinated biphenyls (PCBs) in animals and microorganisms, and the dominant metabolites are shown to be hydroxylated compounds and their conjugates (a review; SUNDSTRÖM et al. 1976). In the case of isopropylchlorobiphenyls, they are metabolized along two major routes; one pathway proceeds by stepwise oxidation of the isopropyl group to chlorobiphenyl carboxylic acid, and the other by hydroxylation of the chlorine substituted phenyl ring (TULP et al. 1977a, 1978). Since metabolism is facilitated by the presence of an alkyl group attached to the aromatic ring, isopropyl-chlorobiphenyls are thought to be less persistent and accumulative than PCBs.

In order to estimate the influence of metabolism on accumulation and excretion of lipophilic compounds, the residual behaviours of 4-chloro-4'-isopropyl-, 3,5-dichloro-4'-isopropyl-, 4,4'-dichloro- and 3,5,3',5'-tetrachloro-biphenyl were compared in mouse and fish by using an internal standard method.

MATERIALS AND METHODS

Materials; 4,4'-Dichlorobiphenyl (m.p., 148-149°C), 3,5,3',5'-tetrachlorobiphenyl (m.p., 167-167.5°C), 4-chloro-4'-isopropyl-biphenyl (m.p., 138-139°C) and 3,5-dichloro-4'-isopropylbiphenyl (b.p., 164-164.5°C (2.5 mmHg)) were synthesized as previously described for a number of similar compounds (SUNDSTRÖM 1973; KOVACIC et al. 1968).

Gas chromatography; For quantitative determination of the chemicals, a Shimazdu 4BM-GC connected with a ECD detector was used. A 0.2 x 200 cm glass column, packed with 3% Silicon OV-17 on Chromosorb W (80-100 mesh) was employed. The flow rate of the carrier gas was 30 ml/min. The temperature program was as follows: the initial temperature of 160°C and increased to 250°C at the rate of 3 °C/min.

High-speed liquid chromatography; A Toyosoda 802 type high speed liquid chromatographic apparatus with an incorporated ultraviolet absorption detector and equipped with a 100 cm Permaphase-ODS (du Pont) column was used in the experiment. The retention time was determined at 17°C, with 70% methanol as the developing solvent. The retention time is related to the partition coefficient of compounds between n-octanol and H_2O (CARLSON et al. 1975).

Accumulation and elimination experiments;

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Mouse; Male mice (five-weeks old) of ICR-JCL® were fed for 25 days on powdered food (Nihon Clea's EC-2), added with 4,4'-dichlorobiphenyl (97 ppm), 4-chloro-4'-isopropylbiphenyl (154 ppm), 3,5-dichloro-4'-isopropylbiphenyl (7.0 ppm) and 3,5,3',5'-tetrachlorobiphenyl (6.1 ppm). Subsequently they were given food which was not contaminated.

A mouse was removed at frequent intervals and the concentration of compounds was measured. They were dissected to expose the digestive tract and other body parts. The body parts were smashed by using a blender with acetone-benzene (1:1, 200 ml) and the suspension was then filtered. This procedure was repeated twice. The collected supernatant was dried over anhydrous sodium sulfate (30 g). The resulting solution was concentrated at 30°C *in vacuo*, and then passed through a silica gel (10 g) column which was subsequently eluted with hexane (200 ml). The eluate was quantitatively analyzed by gas chromatography. The concentration of compounds in the mice was indicated by μg/body-parts weight (g). The recovery was 95% and above.

Fish; 4,4'-Dichloro-, 4-chloro-4'-isopropyl-, 3,5-dichloro-4'-isopropyl- and 3,5,3',5'-tetrachloro-biphenyl were dissolved in a solution containing 1 ppm of Tween 20® in concentrations of 0.05, 0.05, 0.0045 and 0.003 ppm, respectively. 100 l of the solution was put in a glass aquarium and the solution was continuously introduced into the vessel at 400 l/day (25 ± 2°C) for 38 days. Twenty two carp (*Cyrinus carpio*, 29.4 ± 3.3 g) were placed in the vessel and a carp was removed at frequent intervals. For the experiment on elimination process, carp which had been exposed for 38 days were placed in 100 l of uncontaminated water (25 ± 2 °C, exchange rate, 400 l/day) and a carp was removed at frequent intervals.

The concentrations of compounds in the whole body of the carp were determined by the same method as that used for the mice. The concentration of compounds in water was determined by the following method: after the water (200 ml) was treated for 20 h by continuous liquid-liquid extraction with hexane, the solution was dried over anhydrous sodium sulfate (10 g), and concentrated to dryness *in vacuo*. The amount of each compound was determined by using a gas chromatograph. The recovery was 95% or more. The observed concentration of compounds in water was different from the given concentration. The respective mean concentrations obtained in the exposure period of 4,4'-dichloro-, 4-chloro-4'-isopropyl-, 3,5-dichloro-4'-isopropyl- and 3,5,3',5'-tetrachlorobiphenyl were 0.224 (S.D., ± 0.092), 0.256 (0.060), 0.34 (0.20) and 0.17 (0.08) times the given concentrations.

The accumulation factor was calculated as the ratio of the concentration of compounds in fish to the observed concentration in water.

Metabolism experiments; Mice and carp were killed by cervical dislocation. All subsequent manipulations were carried out at 4°C or below. Livers of mice and fishes were homogenized with a Potter-type homogenizer using a Teflon pestle in 4 and 1.5 parts of 1.1% potasium chloride, respectively. The homogenate was centrifuged at 9,000 x g for 20 min. The supernatant was used as the enzyme fraction. The protein contents of the enzyme

fractions of mouse and fish were 21.6 ± 0.3 and 50 ± 10 mg/ml, respectively.

Metabolic activity was measured by the decreased amount of compounds during a 20 min incubation in air at 37°C. Incubation mixtures (15 ml) contained 5 ml of the enzyme fraction, 1 ml of the mixture of DMSO, Tween 80® and H_2O (0.2:1:2) containing 4,4'-dichlorobiphenyl (0.0025 μmol), 4-chloro-4'-isopropylbiphenyl (0.025 μmol), 3,5-dichloro-4'-isopropylbiphenyl (0.5 μmol) and 3,5,3',5'-tetrachlorobiphenyl (0.0025 μmol), 75 mM Tris buffer (pH 7.4), 2.5 mM $MgCl_2$, and an NADPH-generating system consisting of 0.32 mM NADPH, 3 mM glucose-6-phosphate, and 13 units of glucose-6-phosphate dehydrogenase (KATO & GILLETTE 1965; GREB et al. 1975). The buffered NADPH-generating system was incubated for 5 min before adding the enzyme and compounds. The reaction was stopped by adding 20 ml of ethanol. The reaction mixture was extracted with benzene (40 ml, three times). The benzene layer was dried over anhydrous sodium sulfate (10 g). The solution was concentrated and then passed through a silica gel (10 g) column subsequently eluted with hexane (200 ml). The eluate was quantitatively analyzed by using gas chromatography.

Aniline hydroxylase activities of mice and fish under these conditions were 0.12 ± 0.02 and 0.0090 ± 0.0009 μmol/20 min/head, respectively.

RESULTS AND DISCUSSION

The results of hepatic metabolizing activity of substituted biphenyls in mice and fish are summarized in Table 1.

It is clear that chlorobiphenyls were more stable than isopropylchlorobiphenyls in the enzyme fraction of mouse. This result indicates that the metabolism might have been facilitated by the presence of alkyl group attached to the aromatic ring (TULP et al. 1977b). No difference in the stability in the enzyme fraction of fish, however, was found between isopropylchlorobiphenyls and chlorobiphenyls, the reason being that fish have either low or undetectable metabolizing enzyme activity. Indeed, aniline hydroxylase activity found in fish ranged around 1 order of magnitude lower than activity found in mouse.

Daily variations in the accumulation and excretion of substituted biphenyls in mouse and fish are shown in Fig. 1. The accumulation factor and the elimination half-life of each compound are summarized in Table 1.

Fig. 1 shows that the time required to reach the accumulation equilibrium for each compound in mice was shorter than that in fish.

The accumulation factors of these compounds in mice increased in the order: 4,4'-dichlorobiphenyl < 4-chloro-4'-isopropylbiphenyl < 3,5-dichloro-4'-isopropylbiphenyl < 3,5,3',5'-tetrachlorobiphenyl. The accumulation factors in fish increased in the order: 4-chloro-4'-isopropylbiphenyl ≒ 3,5-dichloro-4'-isopropylbiphenyl < 4,4'-dichlorobiphenyl < 3,5,3',5'-tetrachlorobiphenyl. There was a difference in the order of accumulation of these compounds between mice and fish.

The elimination half-lives of these compounds in mice increased in the order: 4,4'-dichlorobiphenyl < 4-chloro-4'-isopropylbiphenyl < 3,5-dichloro-4'-isopropylbiphenyl < 3,5,3',5'-tetrachlorobiphenyl. The elimination half-lives in fish increased in the order: 4,4'-dichlorobiphenyl < 4-chloro-4'-isopropylbiphenyl ≒ 3,5-dichloro-4'-isopropylbiphenyl < 3,5,3',5'-tetrachlorobiphenyl. The order of persistency for these compounds in mice was the same as that in fish.

Basic assumptions of the preliminary pharmacokinetic model are: (1) The equilibrium concentrations of these compounds in the body fluids are proportional the concentrations in food or water; (2) A molecule has to make many partitionings between aqueous phases and fixed organic phases in going through the surface membrance

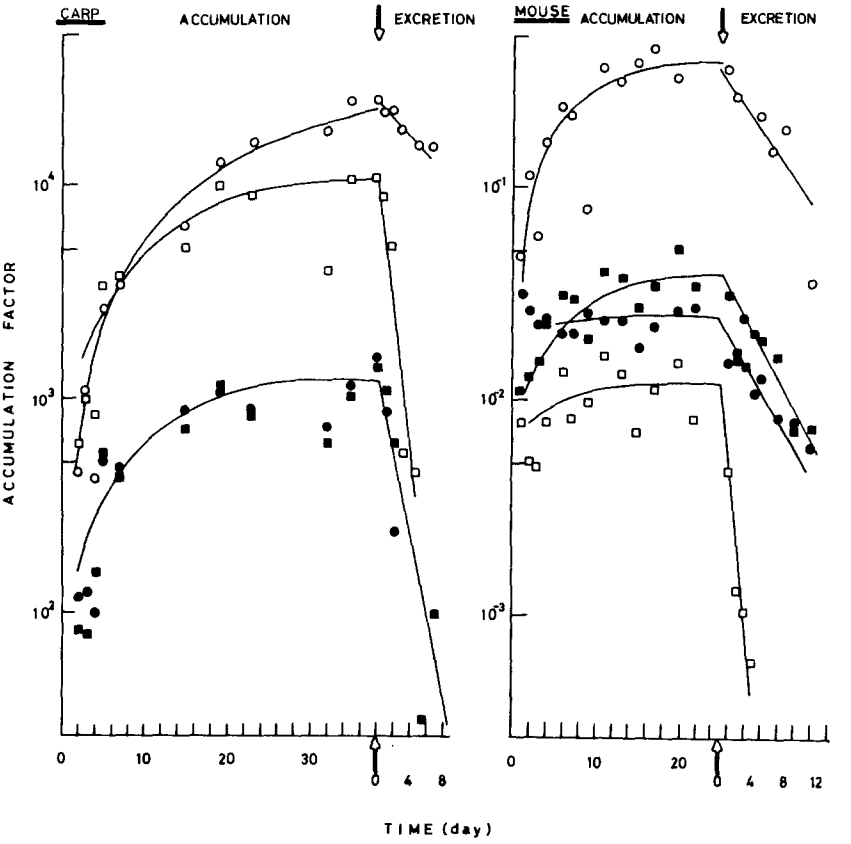

Fig. 1. Concentrations in fish and mice as a function of time.

□, 4,4'-Dichlorobiphenyl; ●, 4-chloro-4'-isopropylbiphenyl; ■, 3,5-dichloro-4'-isopropylbiphenyl; ○, 3,5,3',5'-tetrachlorobiphenyl.

TABLE 1

Reference data concerning the accumulation and excretion of substituted biphenyls in mice and fish.

Substituent	Enzyme activity[a] (µmol/20 min/head)		Accumulation factor		Elimination half-life (day)		Retention time (min)
	mouse	fish	mouse	fish	mouse	fish	
4,4-Dichloro	~ 0	~ 0	0.011	1.4×10^4	1.0	0.8	2.3
4-Chloro-4'-isopropyl	0.019 ± 0.008	~ 0	0.024	1.1×10^3	4.0	1.6	4.8
3,5-Dichloro-4'-isopropyl	0.020 ± 0.006	~ 0	0.043	1.1×10^3	4.4	1.6	11.0
3,5,3',5'-Tetrachloro	~ 0	~ 0	0.43	1.3×10^5 [b]	6.0	7.2	13.0

a) Each value is the average of four experiments.
b) The equilibrium concentration (C_{eq}) was obtained by applying the actually measured values to an empirical equation, $C_t = t/(K + t/C_{eq})$, using the least squares method, where C_t is the concentration in fish at t, t the time (day) and K the constant (ZITKO & HUTZINGER 1976). The accumulation factor is shown by dividing the estimated concentration by the concentration in water.

section. The transportation rates thus vary with the partition coefficient (expressed as n-octanol/water partition coefficient) (HANSCH & FUJITA 1964; SUGIURA et al. 1978; TULP & HUTZINGER 1978; KÜNEMANN & LEEUWEN 1980); (3) The difference in concentration of a compound between the body fluids and the adipose tissues is due to varying compound affinites for the compartments (i.e. partition coefficient) (NEELY et al. 1974; SUGIURA et al. 1976; GEYER et al. 1980); (4) The amount of a compound in the body fluids is negligible compared to the amount found in the adipose tissues (SUGIURA et al. 1979).

The kinetics can be described by three differential equations:

$$dC_d/dt = 0 \quad \text{.... eq. (1)}$$

$$dQ_b/dt = k_1(AC_d - C_b) - k_2C_b - k_3C_b + k_4C_r \quad \text{.... eq. (2)}$$

$$dQ_r/dt = k_3C_b - k_4C_r \quad \text{.... eq. (3)}$$

In these equations, C_b is the concentration of a compound in the body fluids, C_d the concentration in food or water, C_r the concentration in the adipose tissues, Q_b the quantity in the body fluids, Q_r the quantity in the adipose tissues, k_2 the metabolic rate constant for the liver, A the constant which varies with the exposure conditions and k_1, k_3 and k_4 are the transportation rate constants.

The equiliburium concentrations of a compound in the body fluids and the adipose tissues will be given by the following equations;

$$C_{b(eq)} = AC_d/(1 + k_2/k_1) \quad \text{.... eq. (4)}$$

$$C_{r(eq)} = AC_d(k_3/k_4)/(1 + k_2/k_1) \quad \text{.... eq. (5)}$$

The accumulation factors of these compounds should thus vary with the partition coefficient (k_3/k_4) and the ratio of the metabolic rate constant for the liver (k_2) to the transportation rate constant (k_1).

The relationship between the retention time, determined by a high-speed liquid chromatograph, and the value (relative accumulation factor) obtained by dividing the accumulation factor of substituted biphenyls in mice and fish by the accumulation factor of 3,5,3',5'-tetrachlorobiphenyl are shown in Fig. 2.

Fig. 2 shows that, in fish, the proportional correlation was present between the relative accumulation factor of substituted biphenyls but isopropylchlorobiphenyls and the partition coefficient; the relative accumulation factor of isopropylchlorobiphenyls did not follow the partition coefficient. The lower accumulation factor of isopropylchlorobiphenyls might be due to (1) the relatively easier metabolism of isopropylchlorobiphenyls when compared to the corresponding chlorobiphenyls, and (2) the small transportation rate constant (k_1) resulting that the ratio of the metabolic rate constant for the liver (k_2) to the transportation rate constant can be assumed to be not negligible in eq. (5).

The mice had a higher metabolizing enzyme activity than the fish. However, the proportional correlation was observed between the relative accumulation factor of all the substituted biphenyls in the mice and the partition coefficient. It might be that the transportation rate constant (k_1) is greater than the metabolic rate constant for the liver (k_2). The k_2/k_1 value can then be assumed to be negligible in eq. (5). It is known that when

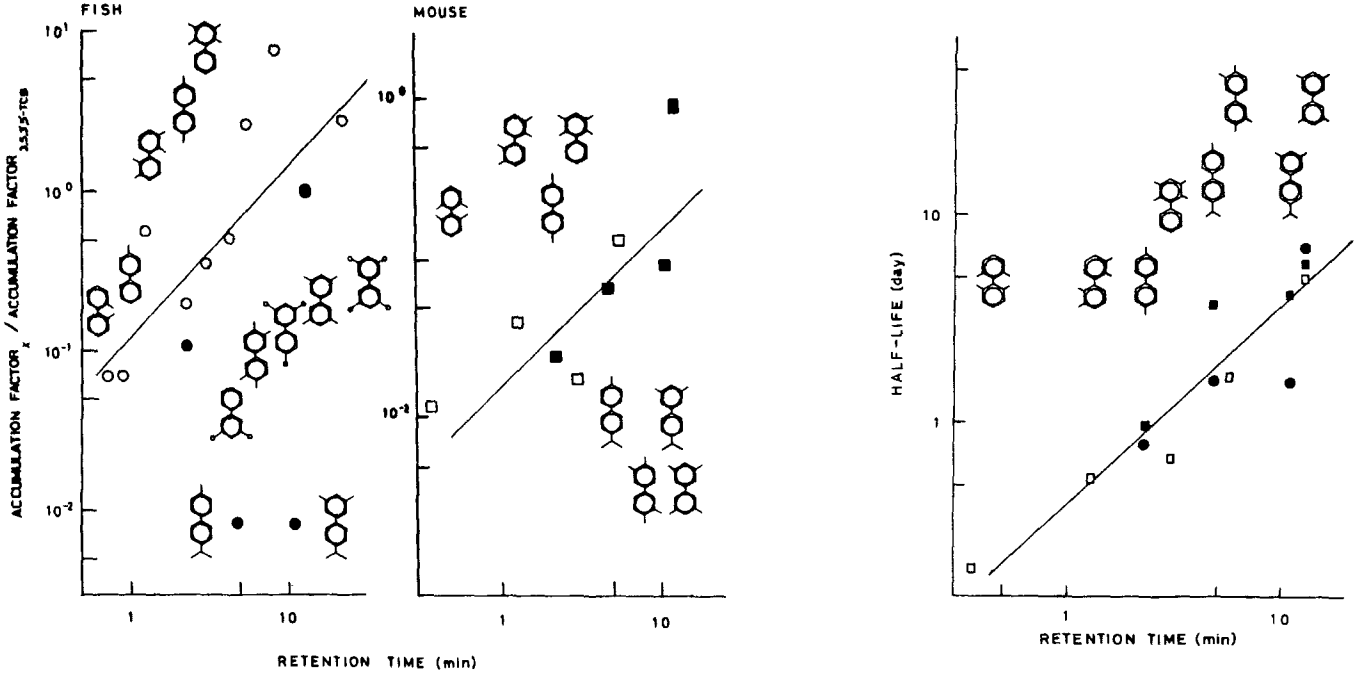

Fig. 2. Relationship between the relative accumulation factor and the retention time determined by a high speed liquid chromatograph (ODS-70% methanol).
●, Carp (Cyrinus carpio); ○, killifish (Oryzias latipes) (SUGIURA et al. 1978); ■, mouse; □, mouse (SUGIURA et al. 1976). Substituent: —, chloro; —o, bromo; —≺, isopropyl.

Fig. 3. Relationship between the elimination half-life in fish and mice and the retention time. Symbols as for Fig. 2.

chlorobiphenyls were fed at a dose rate of between 5 and 100 mg/kg body weight, less than 10% of the amounts fed were excreted in feces (ALBRO & FISHBEIN 1972). This result indicated that the degree of absorption of these compounds is also high.

There was no correlation between the half-life and the rate of metabolism in mice and fish. On the other hand, as shown in Fig. 3, the elimination half-lives of substituted biphenyls were correlated with the partition coefficients. This suggests that the transport rate of substituted biphenyls from the adipose tissues to the body fluids might be the condition limiting rate.

REFERENCES

ALBRO, P.W., and L. FISHBEIN: Bull. Environ. Contam. Toxicol. 8, 26 (1972).

CARLSON, R.M., R.E. CARLSON and H.L. KOPPERMAN: J. Chromatogr. 107, 219 (1975).

GEYER, H., A.G. KRAUS, W. KLEIN, E. RICHTER and F. KORTE: Chemosphere 9, 277 (1980).

GREB, W., K. KLEIN, F. COULSTON, L. GOLBERG and F. KORTE: Bull. Environ. Contam. Toxicol. 13, 424 (1975).

HANSCH, C., and T. FUJITA: J. Am. Chem. Soc. 86, 1616 (1964).

KATO, R., and J.R. GILLETTE: J. Pharmacol. Exptl. Therap. 150, 279 (1965).

KÖNEMANN, H., and K. van LEEUWEN: Chemosphere 9, 3 (1980).

KOVACIC, P., J.F. GORMISH, R.J. HOPPER and J.W. KNAPCZYK: J. Org. Chem. 33, 4515 (1968).

NEELY, W.B., D.R. BRANSON and G.E. BLAU: Environ. Sci. Technol. 8, 1113 (1974).

SUGIURA, K., N. ITO, N. MATSUMOTO, Y. MIHARA, Y. TSUKAKOSHI and M. GOTO: Chemosphere 7, 731 (1978).

SUGIURA, K., N. TANAKA, N. TAKEUCHI, M. TOYODA and M. GOTO: Chemosphere 5, 31 (1976).

SUGIURA, K., N. MATSUMOTO, T. WASHINO, Y. MIHARA and M. GOTO: Chemosphere 8, 365 (1979).

SUNDSTRÖM, G.: Acta Chem. Scand. 27, 600 (1973).

SUNDSTRÖM, G., O. HUTZINGER and S.SAFE: Chemosphere 5, 267 (1976).

TULP, M.Th.M., and O. HUTZINGER: Chemosphere 7, 849 (1978).

TULP, M.Th.M., G.C. van STEIN and O. HUTZINGER: Chemosphere 7, 747 (1978).

TULP, M.Th.M., G. SUNDSTRÖM, J.C. de GRAAFF and O. HUTZINGER: Chemosphere 6, 109 (1977a).

TULP, M.Th.M., G.M. TILLMANNS and O. HUTZINGER: Chemosphere 6, 223 (1977b).

ZITKO, V., and O. HUTZINGER: Bull. Environ. Contam. Toxicol. 16, 665 (1976).