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ACCUMULATION AND EXCRETION OF PCBs IN THE MOUSE+

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Composition of residual PCBs in the adipose tissue of man and animals is reported to be remarkably different from that of technical PCBs. This is supposed to be due to the fact that lower chlorinated compounds are readily excreted and metabolized[1-6]. However, no experimental evidence is available on the relation between structures and accumulation factors. In this paper we report accumulation and excretion of 2,3,5,6-tetrachlorobiphenyl (2,3,5,6-TCB) and 2,4,6,2',4',6'-hexachlorobiphenyl (2,4,6,2',4',6'-HCB) in mice. Now, it has been found that the residues of PCBs depend upon an adsorption - diffusion mechanism in the adipose layer.

EXPERIMENTAL

The PCB-isomers used were synthesized according to the procedures of Hutzinger et al[7]. Male mice (five-weeks old) of ICR-JCL type were fed for 20 days on powdered foods (Nihon Clea's CE-2), added with 0.1, 1.0 and 10 ppm of PCB, and subsequently fed food without contamination. One group consisted of 30 mice, and every two or three mice were sacrificed 5, 10, 15 and 20 days after the administration, and 1, 2, 7 and 15 days after the administration was stopped. They were dissected into the liver, digestive tract and other body parts. These organs were dissolved with a 30% NaOH aq (100 ml, refluxed for 3 h), and with the body parts, 1/5 part by volume of the solution was diluted to 100 ml and treated for 6 h by continuous liquid-liquid extraction with n-hexane. Then the n-hexane was evaporated in vacuo at 30°C, and the concentrated liquid was adsorbed on a column where 1 cm thickness of anhydrous Na_2SO_4 layer was placed at the top of a silica gel column. Then the compound was eluted with 100 ml of

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n-hexane and then concentrated. These compounds were quantitatively analyzed by gas chromatography (a glass column (2 m), OV-17, N_2, 35 ml/min, column temperature: 200°C, an electron capture detector). The yield in the extraction - purification was 70-80%.

RESULTS AND DISCUSSION

Daily variation of adsorption and excretion of 2,3,5,6-TCB and 2,4,6,2',4',6'-HCB are shown in Fig. 1. In equilibrium, there is a linear relationship between the concentration in these feeds and the residues in the body (Fig. 2), i.e. [Accumulation in the body (ppm)] = K [Concentration in the feed (ppm)]^n where K and n are constant.

Determination of the residual amounts after stopping the administration of the contaminated feed indicates the linear relationships shown in Figs. 3 and 4; namely, with 2,3,5,6-TCB, log (residual quantity) is proportional to the period of time, and with 2,4,6,2',4',6'-HCB, log (residual quantity) is proportional to the square root of the period of time.

The period of time required for 2,3,5,6-TCB [in the case where mice were raised on the 10 ppm-feed (Fig. 1, curve a)] was compared with that required for 2,4,6,2',4',6'-HCB [in the case where mice were raised on the 0.1 ppm feed (Fig. 1, curve d)], at the same equilibrium concentration. The comparison shows that the former requires a shorter period of time than the latter.

The above experimental results indicate that accumulation and discharge of PCB depend upon adsorption and diffusion.

Since absorption of PCBs (KC-400) in the intestinal tract of mice is known to be independent of the kind of isomers, PCB concentration in blood is proportional to concentration in food[2]. It was estimated from the result of whole-body autoradiograph that the body part, except the liver and digestive tract, where PCB will accumulate is only the adipose tissue[8].

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The accumulation procedure involves the concentration of PCB in blood through an adsorption phenomenon in the adipose layer, which concentration reaches equilibrium, according to Frendlich-type isothermal adsorption equation. PCB molecules adsorbed on the surface diffuse into the inside of the adipose layer, until the surface concentration and the inside concentration reach equilibrium. The excretion is the procedure where PCB molecules moves from the adipose-layer surface to blood according to the isothermal adsorption equation, and PCB molecules move from the inside of the adipose layer to the surface through diffusion due to the concentration gradient.

In such a mechanism, it is estimated that the adsorption equilibrium in the accumulation procedure of 2,3,5,6-TCB and 2,4,6,2',4',6'-HCB inclines largely to the adsorption side, since these compounds are only slightly soluble in water. It is therefore estimated that adsorption equilibrium on the surface is readily obtained.

The difference between the two compounds in the period of time to reach equilibrium can be considered to depend upon the ease of diffusion of the two compounds, and the difference is attributable to the fact that 2,3,5,6-TCB, which can have a plane configuration, has a smaller cross-sectional area of the molecule than 2,4,6,2',4',6'-HCB, which is sterically hindered and a greater cross-sectional area.

The excretion of 2,3,5,6-TCB is considered to depend upon the inverse procedure of the adsorption phenomenon, because of the linear relationship between log (residual quantity) and the period of time, and therefore diffusion will not be the rate-determining step. The linear relationship between log (residual quantity) and the square root of period of time in the discharge of 2,4,6,2',4',6'-HCB indicates that diffusion is the rate-determining step of this procedure. Extrapolation of this straight line to zero time gives the concentration in the internal layer.

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From this, the relation between adipose-surface concentration and concentration in food, which is proportional to concentration in blood, is given by the following equation:

[Adipose-surface concentration (ppm)] = 0.11 [Concentration in food (ppm)]^{1}

and the relation between adipose-surface and adipose-inside concentrations is given by the following equation:

[Adipose inside (ppm)] = 1.7 [Adipose surface (ppm)]$^{0.8}$.

These two equations indicate that HCB molecules in the inside of the adipose layer have some interaction of chemical bonding with adipose as well as physical interaction. It is supposed that different diffusion coefficients are given because of this fact by different equilibrium concentrations as shown in Fig. 4. It is estimated that the rapid reduction in residual quantity in the early stage is due to the desorption from the surface-adsorption part. Discharge of PCB from the skin of mice[2] can also be well explained by this mechanism.

Now, since adsorbed concentration on the adipose surface is estimated to be close to concentration in the adipose inside, it must be possible to estimate concentrations in accumulation-equilibrium from the constant of adsorption from water to the adipose surface. Fig. 5 shows the relation between adsorption constants (K') of PCB isomers and their retention times determined by a high-speed liquid chromatograph (reversed-phase, adsorber: Permaphase-ODS, developer: 70% methanol). Fig. 5 shows that compounds having the larger halogen number have the greater value of K', and it also shows that this order may be altered by molecular configuration: A compound which has halogen atoms at positions 2,2',6, and 6' of biphenyl to be unable to possess a plane configuration has a smaller value of K' as compared with other compounds of the same halogen number. This fact shows that where PCB-isomers of the same halogen number are continually taken, accumulated amounts of isomers that are unable to have plane configurations are smaller, irrespective of whether or not equilibrium has been reached. This result coincides with data of accumulation in human adipose[3].

Metabolic degradations are considered to affect residual amounts in the body, but the rate of metabolism of PCBs appears not to affect residual quantity

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directly, according to our experiment: Discharge data (Fig. 1,curve c and f) of mice raised on food added with 1 ppm of 2,4,6,2',4',6'-HCB shows an immediate reduction in HCB concentration in the liver, but a mild reduction in the body concentration and from this it is considered that discharge from adipose tissue is the rate-determining step.

These results indicate that accumulation and discharge of PCBs are regulated by the adsorption on the adipose-layer surface and by the diffusion into the inside of tho adipose tissue. It is estimated that the residual mechanism of lipophilic organochlorine compounds such as BHC, DDT, Dieldrin, etc. also depend upon a similar adsorption-diffusion mechanism in the adipose layer. Recently, it has been reported that absorption of DDE through the midge surface depends upon an adsorption-diffusion mechanism, and this report agree with our interpretation[9].

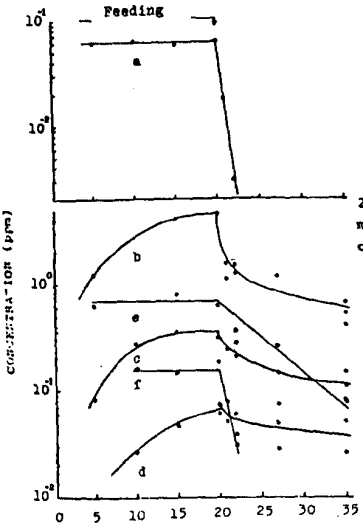


Fig. 1 Residues (ppm) of 2,3,5,6-TCB and 2,4,6,2',4',6'-HCB in the body and liver of male mice during and after feeding on contaminated diets.

a, body, TCB: 10 ppm;
b, body, HCB: 10 ppm;
c, body, HCB: 1.0 ppm;
d, body, HCB: 0.1 ppm;
e, liver, HCB: 10 ppm;
f, liver, HCB: 1.0 ppm.

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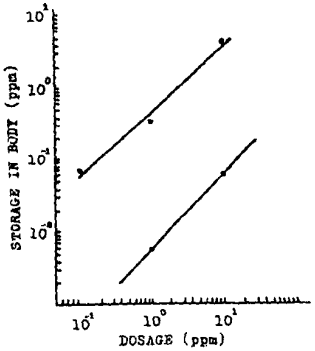


Fig. 2 The relation between residues of 2,4,6,2',4',6'-HCB (upper line) and 2,3,5,6-TCB (lower line) in the body of male mice and the dosage administered.

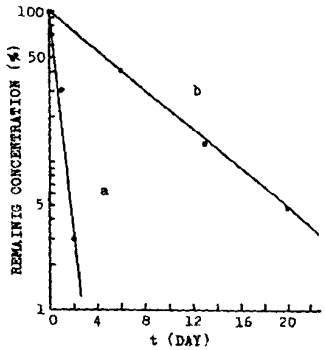
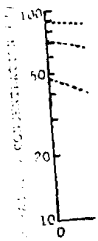
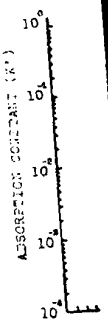


Fig. 3 Decrease of 2,3,5,6-TCB concentrations in the mouse (body part) after stopping administration (a). Decrease of TCB-concentrations in the skin of mice after a single oral dosage of KC-400 is given for the purpose of comparison (b)[2].





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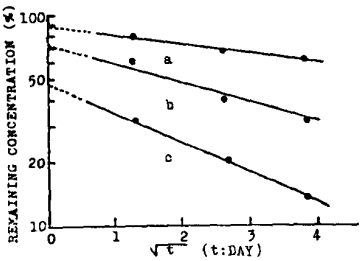


Fig. 4 Decrease of 2,4,6,2',-4',6'-HCB concentrations in the mouse (body part) after stopping administration of the compound.
a,HCB in the diet: 0.1 ppm;
b,HCB in the diet: 1.0 ppm;
c,HCB in the diet: 10 ppm.

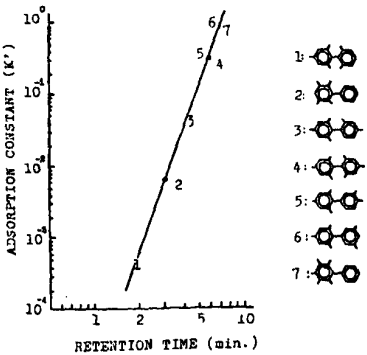


Fig. 5 Structures of PCBs and the adsorption constants determined by retention times using a high speed liquid chromatograph.

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