Metabolism of Diphenyltin Compound in Rat Liver after a Single Oral Administration of Diphenyltin Dichloride

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The metabolism of phenyltin compounds in the rat liver following oral administration of diphenyltin, one of the metabolites of triphenyltin, has been examined. The phenyltin concentrations in the liver were determined by gas chromatography. The concentrations were measured periodically for 72 h after a single oral dose of diphenyltin dichloride was given to rats. We detected not only mono- and diphenyltin but also triphenyltin in the liver, and these compounds were confirmed by gas chromatography–mass spectrometry/selected ion monitoring. No triphenyltin was detected in untreated rat liver to which diphenyltin was added directly. This finding indicates that the diphenyltin compound does not itself decompose and it is not converted into triphenyltin during the analytical procedure. It seems, therefore, that the triphenyltin compound was formed in the liver of the diphenyltin-treated rat by metabolism of diphenyltin dichloride. These results show that part of the administered diphenyltin compound may cause some harmful effects as the triphenyltin compound in the rat, and this must be taken into consideration in toxicological research on diphenyltin.

INTRODUCTION

Organotin compounds have been widely used in the plastics industry, in agriculture, and in the production of antifouling paints (Blunden and Evans, 1990). However, some derivatives of organotin compounds are comparatively highly toxic to mammals (WHO, 1980; Boyer, 1989; Blunden and Evans, 1990), and environmental pollution from these compounds has become a serious problem. In Japan, although the use of butyl- and phenyltin has been regulated since 1988, relatively high levels of these compounds are still being detected in the environment (Ohhira and Matsui, 1990a, 1991). Therefore, a knowledge of the toxicological mechanism and the metabolic fate of organotin compounds in mammals is obviously of considerable importance. As an integral part of our basic research on the toxicology of organotin compounds, we have developed a satisfactory method to determine these compounds. Furthermore, by using this method we have investigated the metabolism of triphenyltin in rats (Ohhira and Matsui, 1990b, 1992).

The present paper describes the metabolism of diphenyltin, one of the metabolites of triphenyltin, in rat livers following a single oral administration of diphenyltin dichloride.

MATERIALS AND METHODS

Chemicals. All chemicals were used without further purification. Triphenyltin chloride was purchased from Tokyo Chemical Industry (Tokyo, Japan). Diphenyltin dichloride and phenyltin trichloride were obtained from Alfa Products (Danvers, MA). Pentylmagnesium bromide (2.0 mol in diethyl ether) was from Aldrich (Milwaukee, WI). Tropolone (2-hydroxy-2,4,6-cycloheptatrienone) was from Sigma (St. Louis, MO). Florisil PR was from Wako (Osaka, Japan), activated at 130 °C for 6 h, after which time 10% (w/w) distilled water was added. The 10% water-deactivated Florisil was rinsed with *n*-hexane before use. *n*-Hexane, benzene, and anhydrous sodium sulfate were of pesticide grade and hydrochloric acid was of trace metal-analysis grade from Wako. All other chemicals were of reagent grade and commercial origin.

Standard Solutions. Standard solutions of each phenyltin compound were prepared at a concentration of 16.8–32.2 mg/10 mL in tetrahydrofuran and stored at 4 °C.

Animals. Male Wistar rats (weighing 180–220 g; Doken, Ibaraki, Japan), fasted for 20–24 h prior to the experiments, were used throughout. The animals were given a single oral administration of 15.4 mg of diphenyltin dichloride as tin/kg of body weight (44.6 mg of diphenyltin dichloride was suspended in 10 mL of sesame oil, and 1 mL/100 g of body weight solution was administered). The control rats were treated with the equivalent volume of sesame oil alone. Four rats in each group were sacrificed at 6, 24, 48, or 72 h after the treatment. The livers were removed and rinsed twice to remove excess blood with an ice-cold medium consisting of 0.25 mol of sucrose, 0.1 mmol of EDTA, and 3 mmol of Tris-HCl (pH 7.4). These samples were kept at −80 °C until analysis.

Determination of Phenyltin Compounds. The phenyltin concentrations in rat liver samples were determined by gas chromatography (GC) according to the technique described previously (Ohhira and Matsui, 1992). Samples for GC were also applied to GC–mass spectrometry (GC–MS), and the standard solutions used for GC–MS were pentylated in the same way as the organic extracts from tissue samples.

Apparatus. For measuring phenyltin compounds, a Hewlett-Packard (Avondale, PA) Model 5890 A GC equipped with a flame photometric detector (FPD, Sn-mode filter 610 nm), an automatic sampler HP Model 7673, and a 12 m × 0.2 mm i.d. HP Ultra-1 capillary column (100% dimethylpolysiloxane gum, 0.33-μm film) were used. Chromatograms were recorded on an HP Model 3396 integrator in the peak height mode.

For identification of phenyltin compounds, HP Model 5890 A GC was connected to a 5970 B mass selective detector (quadrupole mass filter). A GC–MS was operated using HP 59970 C MS Chemistation computer software. The capillary column, an HP Ultra-1 (25 m × 0.2 mm i.d., 0.33-μm film), was directly interfaced to the mass spectrometer ion source. Electron impact (EI) mass spectra were recorded at an ionization potential of 70 eV. The injector and detector temperatures were maintained at 260 and 280 °C, respectively. The column temperature was initially 60 °C, held for 2 min, increased to 160 °C at 40 °C/min, and finally increased from 160 to 260 °C at 20 °C/min. Helium was the carrier gas with a column head pressure of 30 psi (an average linear velocity of 36 cm/s).

RESULTS AND DISCUSSION

The GC-FPD has been used to separate and determine the organotin species in biological materials. The method separated and detected phenyltin compounds and gave high sensitivity (Ohhira and Matsui, 1990b, 1992). Al-

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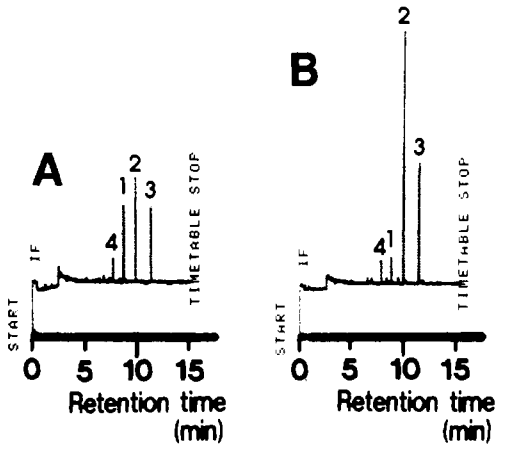

Figure 1. Gas chromatograms of pentyltin derivatives. Tin compounds were extracted from control rat liver homogenate spiked with mono-, di-, and triphenyltin (the amount of each phenyltin compound was 0.2 μg as tin/0.25 g as tissue) (A) and extracted from liver homogenate obtained from a rat at 24 h after a single oral administration of diphenyltin dichloride (B). Peaks: 1, tripentylphenyltin; 2, dipentyldiphenyltin; 3, pentyltriphenyltin; 4, unknown (probably tetrapentyltin).

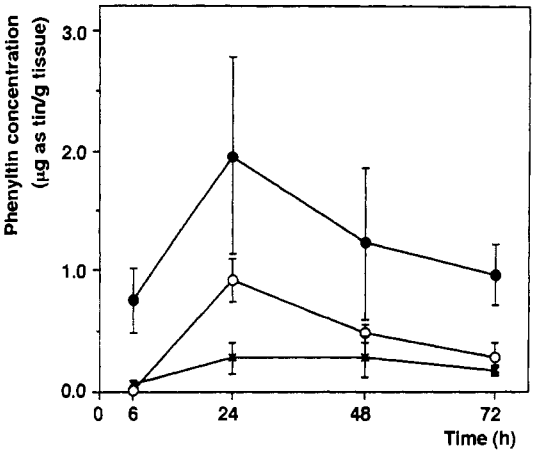

Figure 2. Time course of distribution changes in phenyltin concentrations in rat livers after a single oral administration of diphenyltin dichloride. (×) Mono-, (●) di-, and (○) triphenyltin. One point represents the mean of four rats ± SD.

though the metabolism of triphenyltin has been rather extensively studied, no research to date has examined the metabolism of diphenyltin (Blunden and Evans, 1990). Therefore, we decided to apply the GC-FPD method to the study of the metabolism of diphenyltin in rats.

The metabolism of phenyltin compounds in the liver was observed periodically for 72 h after a single oral administration of diphenyltin dichloride. Figure 1A illustrates a typical chromatogram of phenyltin compounds, which were obtained from liver homogenates spiked with mono-, di-, and triphenyltin, and Figure 1B shows a chromatogram of phenyltin compounds extracted from a rat liver sample at 24 h after administration of diphenyltin dichloride. As shown in Figure 1, diphenyltin and its metabolites, tri- and monophenyltin, were detected in the rat liver sample dosed with diphenyltin dichloride.

Figure 2 shows the time course of the concentrations of diphenyltin and its metabolites in the livers. After the levels of di- and triphenyltin in the livers reached the maximum amounts about 24 h following administration of diphenyltin, it showed a moderate decrease. The amount of triphenyltin approximated half those of diphenyltin at 24 and 48 h after administration. The levels

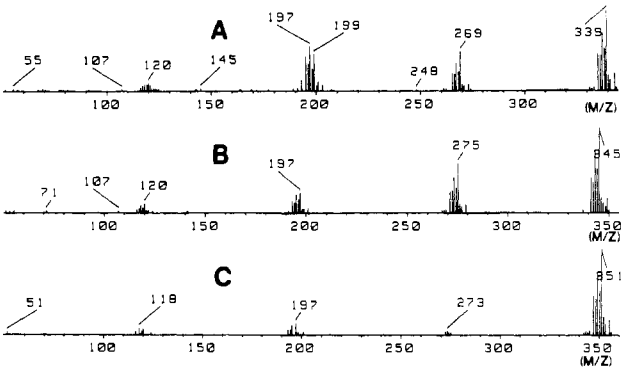

Figure 3. EI mass spectrum of pentyltin derivatives. Tin compounds were obtained with standard solution of mono-, di-, and triphenyltin. (A) Tripentylphenyltin; (B) dipentyldiphenyltin; (C) pentyltriphenyltin.

of monophenyltin in the livers were lower than those of di- and triphenyltin throughout the time period studied.

The metabolism in vivo in rats of triphenyltin has been studied by Freitag and Bock (1974), and it was found that the degradation of triphenyltin chloride proceeds via diphenyltin and monophenyltin to inorganic tin. Additionally, Kimmel et al. (1977) have examined the breakdown of triphenyltin acetate in rats and found a similar breakdown pattern of this triaryltin compound. However, we obtained an interest finding that triphenyltin was detected in the rat liver after administration of diphenyltin dichloride. Further confirmation was performed by GC–MS spectra. GC–MS/selected ion monitoring (SIM) is a powerful method for trace determinations of target analysis (Reiner and Clement, 1990). We used this method to confirm diphenyltin and its metabolites in rat liver samples because these concentrations in the livers were at relatively low levels. Figure 3 shows EI mass spectra obtained with a standard solution of three pentylated phenyltin compounds. The molecular ion cluster is not observed, but each typical tin cluster is evident as the loss of one pentyl group. The specific major ions for each organotin compound were detected, and respective retention times are as follows: monophenyltin (Figure 3A), m/z 339, 10.4 min; diphenyltin (Figure 3B), m/z 345, 11.5 min; triphenyltin (Figure 3C), m/z 351, 13.1 min. The mass spectrum of three phenyltin compounds monitored by SIM and the retention times were observed in the liver sample at 24 h after oral administration of diphenyltin dichloride (Figure 4). In addition, we examined the substance of peak 4 (Figure 1, unknown peak) with the GC–MS technique and detected a major mass peak of m/z 333. This suggests that the substance is a tetrapentyltin derived from inorganic tin.

To exclude the possibility that triphenyltin was produced from diphenyltin during the analytical procedure, we performed the following experiment. Diphenyltin dichloride was added directly to liver homogenates from untreated rats and analyzed according to the same procedure as that of the rat liver samples administered with diphenyltin dichloride. No triphenyltin was detected from the sample of the above experiment as shown in Figure 5. This finding indicates that diphenyltin does not itself decompose and it is not converted into triphenyltin by the analytical procedure. Therefore, the appearance of triphenyltin in the livers of the diphenyltin-treated rats may be due to in vivo production by metabolism of diphenyltin dichloride.

These results show that part of the administered diphenyltin has potential harmful effects as triphenyltin in rats. It is well-known that triorganotin groups appear

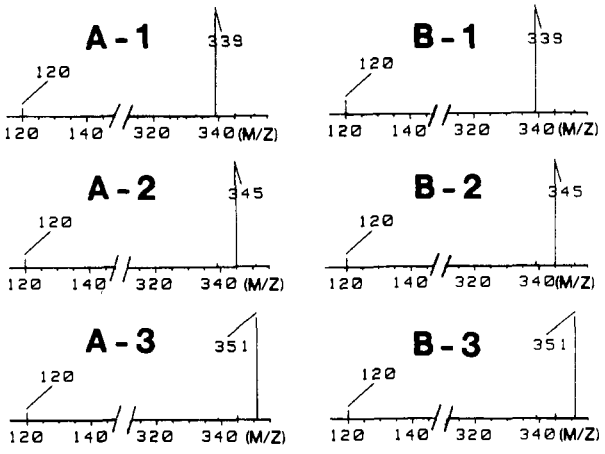

Figure 4. SIM mass spectrum at m/z 120, 339, 345, and 351 of pentyltin derivatives. Tin compounds were obtained with standard solution of mono- (A-1), di- (A-2), and triphenyltin (A-3) and extracted from liver homogenate obtained from a rat at 24 h after a single oral administration of diphenyltin dichloride (B-1, B-2, B-3). Retention times: A-1, B-1, 10.4 min for monophenyltin; A-2, B-2, 11.5 min for diphenyltin; A-3, B-3, 13.1 min for triphenyltin.

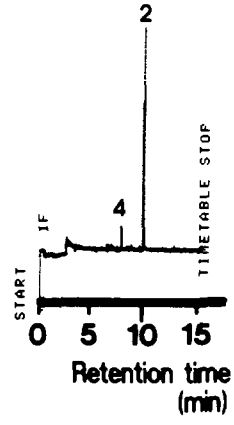

Figure 5. Gas chromatogram of pentyltin derivatives. Tin compounds were extracted from control rat liver homogenate spiked with diphenyltin dichloride (the amount of diphenyltin was 0.4 μg as tin/0.25 g as tissue). Peaks (the same as in Figure 1): 2, dipentyl diphenyltin; 4, unknown (probably tetrapentyltin).

to be more toxic than mono- and disubstituted ones (Blunden and Chapman, 1986; Winship, 1988). Thus, the biological production of triphenyltin from diphenyltin in rats must be taken into consideration in toxicological research on diphenyltin, and it is necessary to elucidate the mechanism of this finding with further studies.

ACKNOWLEDGMENT

We gratefully acknowledge the help of Kunio Suzuki, Mamoru Yotoriyama, and Hideo Yatabe, Tochigi Prefectural Institute of Public Health, in the GC–MS measurement and Katsuko Takakuwa, Nagako Nemoto, and Kiyomi Yamada for valuable technical assistance.

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Received for review October 13, 1992. Accepted January 26, 1993.