Bull. Environ. Contam. Toxicol. (1990) 44:294–301

Bulletin of Environmental Contamination and Toxicology

Simultaneous Determination of Triphenyltin and Its Metabolites, Mono- and Diphenyltin, in Biological Materials by Capillary Gas Chromatography

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Organotin compounds are widely used as agricultural pesticides, antifoulants, and stabilizers for vinyl chloride polymers. The world-wide production of organotin chemicals has risen, over the last 30 years, from under 5,000 tons in 1955 to at least 35,000 tons in 1985 (Blunden and Chapman 1986). The increased production and consumption of organotin chemicals are due primarily to its wide range of industrial applications. Therefore, it is of utmost importance to determine the environmental distribution of organotin pollutants and their potential harmful effects on humans (Matsui et al. 1983; Mochida et al. 1988). As an integral part of our basic research on the toxicology of organotin compounds, we have devoted effort to develop an analytical method for tributyltin and its metabolites. By using capillary gas chromatography (GC) with flame photometric detection (FPD), we have obtained satisfactory results (Ohhira and Matsui 1989a,b).

This paper describes the simultaneous determination of triphenyltin and its metabolites, mono- and diphenyltin, in biological materials by using GC-FPD. We have applied this procedure to the study on the metabolism of triphenyltin in rats.

MATERIALS AND METHODS

All chemicals were used without further purification. Triphenyltin chloride and butylmagnesium chloride (2.0 *M* in tetrahydrofuran) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Diphenyltin dichloride and phenyltin trichloride were obtained from Alfa Products (Denvers, MA, U.S.A.). Tropolone was from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). The other reagents and solvents used were of reagent grade.

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Each organotin compound was initially dissolved in tetrahydrofuran (THF) and either diluted with benzene (containing 0.05% of tropolone) to prepare a standard solution or with distilled water to prepare a fortification solution. The final THF concentration of both the benzene and water solutions was under 0.5%.

A tissue sample weighing less than 2.0 g (wet wt.) was macerated in 10 mL of distilled water. The macerate was placed in a centrifuge tube with a glass stopper, and 1.5 g of sodium chloride and 0.5 mL of concentrated hydrochloric acid (35%) were added to the tube. The mixture was extracted twice with 10 mL of benzene each time (containing 0.05% of tropolone) by agitating for 20 min in a mechanical shaker. After each shaking, the mixture was centrifuged for 10 min at 3000 rpm. Each benzene extract was removed. The benzene extracts were combined and dried with 0.5 g of anhydrous sodium sulfate. The benzene extract was transferred to a separatory funnel, and 1 mL of butylmagnesium chloride was added. The reaction mixture was slowly stirred for 20 min at room temperature and subsequently extracted with 25 mL of distilled water. The organic layer was separated by centrifugation for 30 min at 3000 rpm and then evaporated just to dryness after drying with 0.5 g of anhydrous sodium sulfate. The residual material was dissolved in 10 mL of toluene for GC. A 0.5-μL volume of the sample solution was injected into the GC. The standard solution used for calibration curves was butylated in the same way, but without the extraction step.

For a single oral administration to male rats (Wistar-derived, weighing 200-230 g; Doken, Ibaraki, Japan), triphenyltin chloride suspended in sesame oil (5 mg/mL) was used. The dose level used was 50 mg triphenyltin chloride per kg of body weight. The control rats were treated with the equivalent volume of sesame oil. The rats were killed 6, 12, 24, 48, 72, 120 or 240 hr after the treatment. The mono-, di- and triphenyltin contents in the liver and kidney were determined. The tissues of the control rats were used for analyte recovery-studies.

A GC, model 5890A (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a FPD was operated in the sulfur mode (filter 393 nm). The column was a Ultra 1 capillary column (12.5 m x 0.2 mm, 100% dimethyl polysiloxane Gum, 0.33-μm film thickness ; Hewlett-Packard). The other GC conditions used are given in Table 1.

Table 1. GLC conditions.

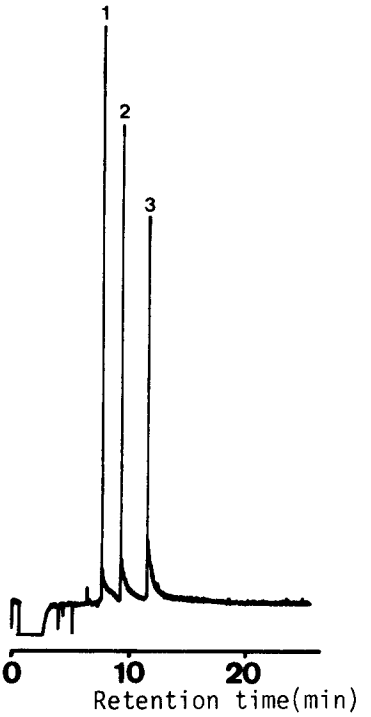
Temperature : inlet;220°C, detector;250°C, oven;80°C (2 min)-(30°C/min)→150°C-(20°C/min)→200°C-(10°C/min)→230°C (20 min)
Air flow mode : H;175 mL/min, air;65 mL/min
Carrier gas : He;2 mL/min Purge gas : He;50 mL/min
Make up gas : N;28 mL/min
Inlet type : splitless (purge time 2 min)
Detector : FPD (S filter 393 nm)

RESULT AND DISCUSSION

The photometric detection mechanism is attributed to light emission of the excited organotin species in the detector flame. The response is strongly dependent on the flame conditions, and the hydrogen-rich flame was found to enhance selectivity as well as the shape of the GC signals (Aue and Flinn 1977). This was consistent with our previous experimental results (Ohhira and Matsui 1989b). Therefore, the hydrogen flow-rate for the flame condition was raised up to 175 mL/min and conversely, the air flow-rate was decreased to 65 mL/min (see Table 1). As a constant injection technique was required for the reproducibility of GC results, the needle was left in the inlet for 10 sec after injection.

The variety of derivatization techniques such as hydrogenation (Sullivan et al. 1988), methylation (Sasaki et al. 1988), and ethylation (Müller 1987) have been applied to the determination of organotin compounds. However, the volatile derivatives are lost during routine concentration procedures involving removal of solvents. To overcome the volatility problem, an n-butyl derivative was prepared for analysis of triphenyltin compounds in our previous work (Ohhira and Matsui 1987). The n-butyl derivatives were sufficiently non-volatile to prevent the loss during solvent removal, yet sufficiently volatile for use in analyses by GC; also the derivatising reagent is readily available. Fig.1 shows the gas chromatograms obtaind with phenyl tributyltin (88 pg as tin), diphenyl dibutyltin (83 pg as tin) and triphenyl butyltin (94 pg as tin). The chromatogram shows good separation of the three tin compounds and sensititive detection. The detection limits of phenyltin compounds were in the 7-9 pg range as tin.

Simultaneous extraction of mono-, di- and triphenyltin from tissues with various solvents was examined. Triphenyltin with a high solubility in organic solvents



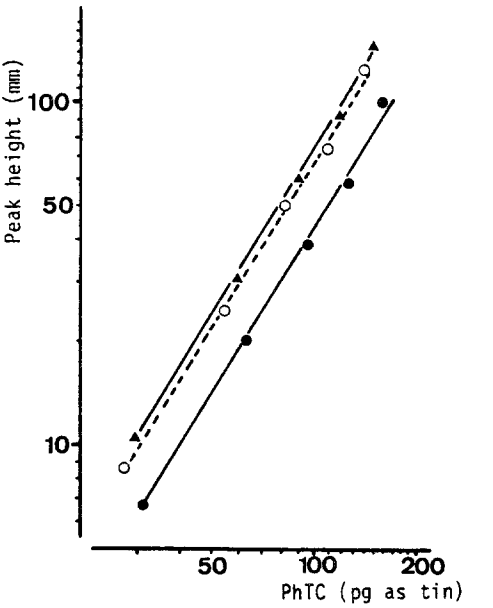


Figure 2. Calibration curves of PhTC. ●:triphenyltin, ○: diphenyltin, ▲:monophenyltin.

Figure 1. Gas chromatogram of phenyltin compounds (PhTC). Peaks: 1;phenyl tributyltin, 2;diphenyl dibutyltin, 3; triphenyl butyltin.

Table 2. Recovery of PhTC added to 2.0 g of rat tissues.

tin species	range of addition (μg of tin compounds)	average recoveries (%±SD) n=10 liver	kidney
$Ph\ Sn^{3+}$	1.5 - 7.5	70.9(±1.8)	70.3(±2.6)
Ph_2Sn^{2+}	1.6 - 7.9	73.1(±1.0)	73.3(±1.0)
Ph_3Sn^{+}	2.0 - 10.2	70.3(±2.2)	71.5(±3.2)

could be easily extracted by using low-polarity and non-polar solvents, such as benzene, n-hexane, diethyl ether, and ethyl acetate. However, the use of organic solvent alone was not suitable for simultaneous extraction, because mono-, and diphenyltin are slightly polar. The use of tropolone as a complexing reagent in the extraction of phenyltin species from aqueous solutions into organic solvents has been studied in great detail (Freitag and Bock 1974). Thus, extraction

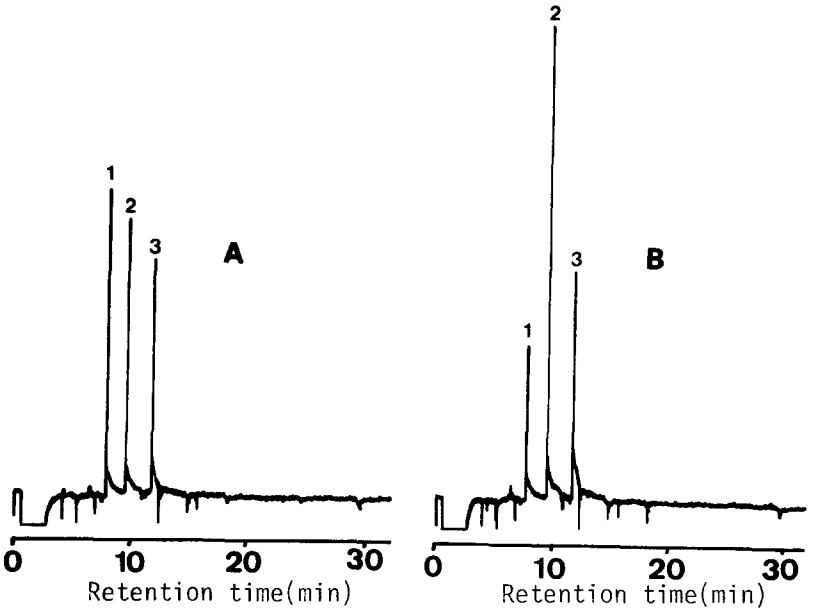


Figure 3. Gas chromatograms for a liver sample fortified with PhTC (A) and kidney sample after 24 hr from a rat dosed with triphenyltin chloride (B). Peaks:1; phenyl tributyltin,2;diphenyl dibutyltin,3;triphenyl butyltin.

with benzene in the presence of 0.05% of tropolone was used. The recoveries of mono-, di- and triphenyltin chlorides added to 2 g of homogenized liver and kidney of rats were evaluated. The results are shown in table 2. The average recoveries were 70-71% for monophenyltin, 73% for diphenyltin and 70-72% for triphenyltin. There was no difference in the recoveries from the liver and kidney. The values of the yield were relatively low;around 70%. However, the procedure consists of a simple extraction system and is able to determine mono-, di- and triphenyltin compounds simultaneously. Both the analytical sensitivity and reproducibility of the yields are considered quite satisfactory.

The calibration data for each phenyltin compound are shown Figure 2. The curves (log-log) exhibit good linearity for the amount of tin versus peak-height at levels of 25 - 160 pg as tin.

The gas chromatographic method was also applied to the study on the metabolism in the liver and kidney of rats orally administered with triphenyltin chloride. Figure 3 illustrates typical chromatograms obtained from (A) liver samples fortified with mono-, di-,

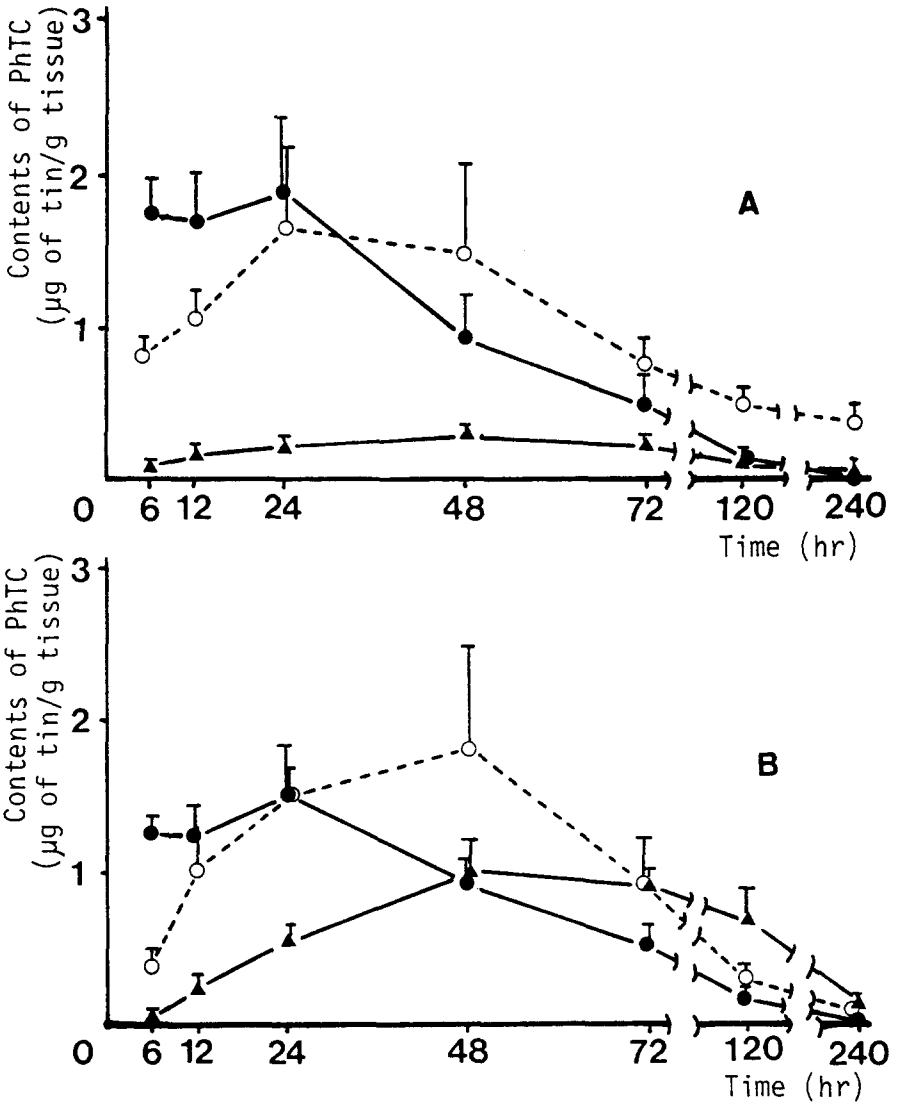


Figure 4. Time course of PhTC contents in liver (**A**) and kidney (**B**) of rats after a single oral administration. ●:triphenyltin, ○:diphenyltin, ▲:monophenyltin. One point represents the mean of four rats±SD.

and triphenyltin compounds and (B) a kidney sample from a rat dosed with triphenyltin chloride. These results indicate that there are no serious interferences from the sample matrix and they are separated well. No phenyltin compounds were detected in the tissues of the control rats. In the recovery studies from the mixture of tissue homogenates and tin com-

pounds, therefore, the individual calibration curves were used independently to determine each phenyltin compound in tissues. Figure 4 shows the time course of the distribution of triphenyltin and its metabolites in the liver and kidney. Triphenyltin levels in the liver and kidney reached the maximum amounts 24 hr after administration. The levels increased slightly after dosing and rapidly decreased after 24 hr. On the other hand, the periods showing maximum contents of mono- and diphenyltin in the tissues were further delayed, compared to that of triphenyltin. Mono- and diphenyltin levels in the kidney remained relatively higher than those in liver. This result suggests that the phenyltin compounds in liver are metabolized rapidly. In the similar study of tributyltin (Ohhira and Matsui 1989b), the concentrations of its metabolites were distinctly high in liver and kidney. The appearance of the metabolites from tributyltin seems to be somewhat faster than those from triphenyltin. This implies that triphenyltin tends to be metabolized slower in both organs, compared to tributyltin.

The method described here is a rapid, accurate means for the determination of phenyltin compounds in biological materials. We think this method will be widely applicable for the studies of the other organic tin compounds.

Acknowledgments. The authors are grateful to Mrs. Katsuko Takakuwa and Miss Tahori Sekiya in our laboratory for technical assistance.

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Received June 7, 1989; accepted June 20, 1989.