Journal of Chromatography, 216 (1981) 209–217
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,109

SIMULTANEOUS DETERMINATION OF TRIALKYLTIN HOMOLOGUES IN BIOLOGICAL MATERIALS

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(First received October 7th, 1980; revised manuscript received June 1st, 1981)

SUMMARY

Taking advantage of the high sensitivity of an electron capture detector to alkyltin halides, an analytical method has been developed for the simultaneous determination of trialkyltin homologues in biological materials. Trialkyltins were purified as chlorides from tissues by simultaneous extraction with hydrochloric acid and ethyl acetate, replacement of the extraction solution with *n*-hexane and stepwise elution with *n*-hexane–ethyl acetate on a silica gel column. Alternatively, gas chromatographic analysis was carried out on 20% DEGS-HG at temperatures from 100 to 120°C. Detection limits reached $1 \cdot 10^{-12}$ g for trialkyltin chlorides. The recoveries of trialkyltins added to various tissues at the 50-pmole level ranged from 97 to 106%. By *in vivo* studies, it was confirmed that this method is rapid, sensitive and applicable to biomaterials containing more than 1 ng trialkyltins per gram of tissue.

INTRODUCTION

The lower trialkyltin compounds, at least up to tributyltin, exhibit fungicidal activity and have been used mainly in biocidal applications. These homologues are also more toxic to mammals than mono-, di- and tetraalkyltin compounds, exerting a specific effect on the central nervous system[1,2].

In the course of comparative studies on the toxicity to animals of trialkyltin homologues, we found that there is a need for suitable analytical techniques for the

0021-9673/81/0000–0000/$02.50

determination of trialkyltins simultaneously present in biological materials. Recently, a variety of analytical methods have been applied to the determination of organotin compounds in aqueous environments. Mono-, di-, and trimethyltin compounds in natural water and human urine were analyzed by conversion into the corresponding volatile hydrides with sodium borohydride followed by detection of the hydrides by a hydrogen–air flame emission detector[3]. Further, seven alkyltin hydrides were detected by atomic absorption spectrometry after conversion of their halides into hydrides with sodium borohydride and separation on the basis of their boiling points; mono- and dimethyltin and mono- and dibutyltin compounds in some environmental waters were also analyzed[4]. Butyltin series in water have been analyzed by extraction with benzene, chloroform or methylene chloride, conversion into the corresponding butylmethyltin compounds with methylmagnesium chloride and detection of the resulting tetrasubstituted alkyltins by gas chromatography–mass spectrometry (GC–MS)[5]. On the other hand, phenyltin series in water were detected by electron-capture GC after extraction from water with dichloromethane and conversion into their hydride derivatives with lithium aluminium hydride[6]. Cyclohexyltin series in fruit crops were detected as the bromide derivatives by extraction with benzene, derivatization with aqueous hydrobromic acid, silica gel clean-up and Coulson conductivity-detector GC[7].

Although these methods have good sensitivity and excellent detection limits in the range of $1 \cdot 10^{-7}$–$1 \cdot 10^{-9}$ g, none gives optimum analytical conditions for the simultaneous determination of triethyl-, tripropyl- and tributyltin compounds in biomaterials. Furthermore, because of the lower recoveries in derivatization and separation of alkyltins, most of these methods are difficult to apply directly to our studies on the behaviour of trialkyltin compounds in mammals.

In this work, we have examined the GC separation of trialkyltin compounds and their purification from biological materials, and established a rapid electron-capture GC procedure for the simultaneous determination of trialkyltins in various biological materials.

EXPERIMENTAL

Reagents

Trimethyltin chloride (Me_3SnCl), triethyltin chloride (Et_3SnCl), tripropyltin chloride (Pr_3SnCl) and tributyltin chloride (Bu_3SnCl) were obtained from the Aldrich (Milwaukee, WI, U.S.A.). Their purities were not less than 98%. When necessary, the compounds were purified by distillation or by silica gel column chromatography (see Fig. 1). Other reagents included special grade materials and organic solvents such as silica gel (No. II A, 100–200 mesh; Nakarai, Tokyo, Japan), *n*-hexane and ethyl acetate (each from Wako, Tokyo, Japan).

Gas chromatography

The instrument was a Shimadzu Model GC-6AM gas chromatograph equipped with an electron-capture detector (ECD). The glass tube (100 cm × 3 mm I.D.) was packed with 20% DEGS-HG on a Chromosorb W (80–100 mesh) support. Other conditions are described in Fig. 2.

Preparation of trialkyltin compounds from tissues

An outline of the sample preparation for trialkyltin compounds in tissues is shown in Fig. 1. A sample of tissue weighing less than about 5.0 g wet weight was homogenized in 10 ml water. Hydrochloric acid (8 ml) was carefully added to the homogenate and the contents of the tube were mixed thoroughly and allowed to stand for 5 min. Ethyl acetate (20 ml), NaCl (2 g) and a suitable amount of internal standard were added and the trialkyltins were extracted by shaking for 5 min. After centrifuging for 10 min at 1000 *g*, the upper ethyl acetate layer was placed into a 30-ml pear-shaped flask. This ethyl acetate extraction procedure was repeated twice.

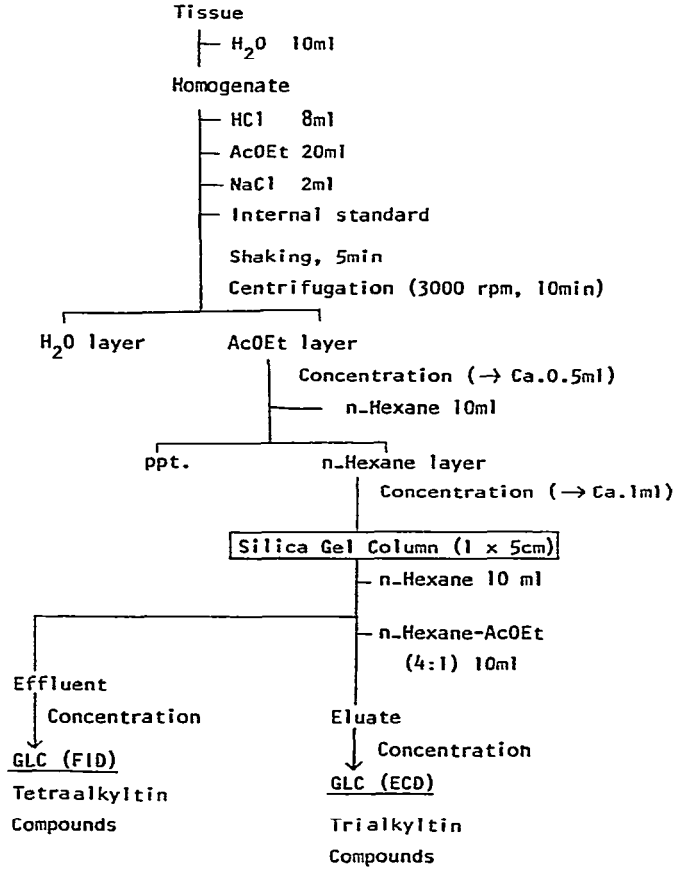

Fig. 1. Preparation of trialkyltin compounds from tissues. AcOEt = Ethyl acetate; FID = flame ionization detector.

The combined ethyl acetate layers were then concentrated under reduced pressure at about 20°C to 0.5–1.0 ml. *n*-Hexane (10 ml) was added to the concentrated solution and the resulting precipitate was removed by centrifugation. The hexane supernatant was transferred to another flask and again concentrated to 0.5–1.0 ml, followed by passage through a silica gel column (5 × 1 cm) conditioned by washing with *n*-hexane. The column was first washed with 10 ml *n*-hexane and then eluted with 10 ml

hexane–ethyl acetate (4:1). A 1–2-μl volume of the eluate was injected directly into the gas chromatograph under suitable conditions, if necessary after concentrating to appropriate volume. Especially in the case of micro-analysis, the eluate was concentrated to 100 μl, and 5–10 μl of the concentrated solution were injected.

Animals

Groups of four male rats (Wistar-derived, weighing 170–200 g) for a single oral administration, and groups of three male rats (Wistar-derived, 300–350 g) for repeated oral and subcutaneous administrations, were used. For addition tests, male rabbits (Japanese white rabbit, 2.0–2.3 kg; Nippon Bio-supp Center, Tokyo, Japan) were used.

Oral treatment

For a single oral administration to rats, tributyltin chloride was dissolved in salad oil. The dose level used was 6.5 mg tributyltin chloride per kilogram of body weight. The rats were killed 3, 6, 12, 24, 48 or 168 h after treatment. Liver, kidney, brain, spleen, red cell and serum samples were prepared for GC analysis of tributyltin chloride (Fig. 4).

For repeated oral administrations to rats, a mixture of triethyltin chloride, tripropyltin chloride and tributyltin chloride dissolved in salad oil was administered five times, once every 12 h. The dose level used was 3.0 mg per kg body weight for each trialkyltin chloride. The animals were killed 4 h after the last treatment. Tissue slices of gastrointestinal tracts, liver, kidney, brain and a sample of whole blood were prepared for analysis of trialkyltin homologues (Fig. 6). The gastrointestinal tracts were thoroughly rinsed in saline, and then in 70% ethanol. A small intestine was cut into three separate parts, duodenum, jejunum and ileum.

Subcutaneous treatment

For repeated subcutaneous administrations, a mixture of triethyltin chloride and tributyltin chloride dissolved in salad oil was injected to rats three times, once every 2 h. The dose level used was 10.0 mg per kg body weight for each trialkyltin chloride. The rats were sacrified 2 h after the last injection. The liver, brain, small intestine and their contents were prepared for analysis of trialkyltin chlorides (see Fig. 5).

RESULTS AND DISCUSSION

Selection of analytical conditions

GC. Using the ECD, the resolution of trialkyltin compounds was examined on various stationary liquid phases and solid column supports. Adsorption of trialkyltins on the exposed sites of the support surface could be overcome by baking of Chromosorb (80–100 mesh) at 300°C for 5 h, washing with acid and alkali, drying at 50°C and treatment with dimethyldichlorosilane. It was possible to elute trialkyltin chlorides through weakly polar liquid phases such as DEGS, SE-52 and XF-1105. For example, the complete separation of three trialkyltin chlorides was achieved on 20% DEGS-HG within 10 min at increasing temperatures from 100 to 120°C (Fig. 2B) or within 6 min under isothermal conditions at 120°C (Fig. 2A). This column also gave suitable peak shapes and sensitivity. Polar stationary liquid phases such as

Carbowax 300M and PEG 20M often gave tailing and broad peaks, and non-polar liquid phases such as OV-1, OV-101, SE-30 and squalane could not be used because of the adsorption and decomposition of trialkyltin chlorides. The retention time of trialkyltin chlorides was affected by the polarity of liquid phases. In weakly polar liquid phases, the chlorides were separated according to their molecular weights and boiling points. Other conditions are shown in Fig. 2.

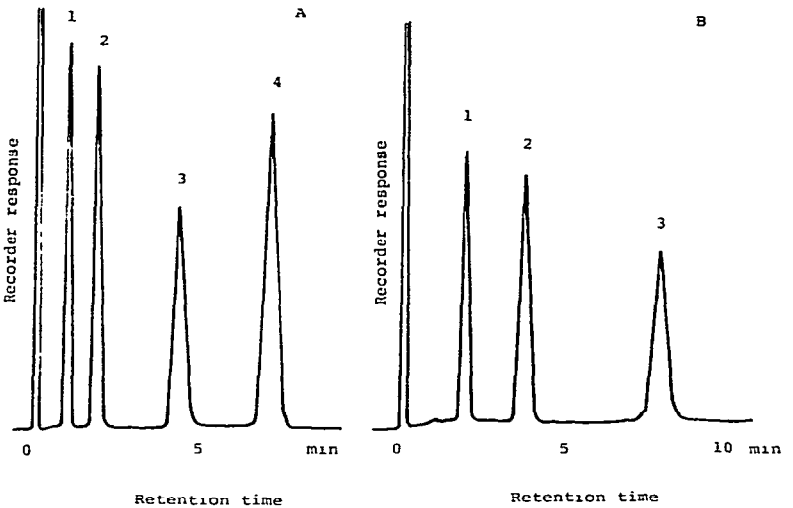

Fig. 2. Gas chromatograms of trialkyltin compounds. Column: 20% DEGS-HG on Chromosorb W (80–100 mesh), 1.0 m × 3 mm I.D. Temperatures: A, 120°C; B, programmed from 100 to 120°C at 2°C/min; ECD, 180°C; injection port, 180°C. Carrier gas: nitrogen at 90 ml/min (A) and 70 ml/min (B). Sensitivity: 100. Range: 0.8 V. Peaks: 1 = Et_3SnCl; 2 = Pr_3SnCl; 3 = Bu_3SnCl; 4 = CH_3HgCl (internal standard).

Internal standard. To minimize errors due to mechanical losses, an internal standard was added to the crude sample before preparation. Tripropyltin chloride was used for the analysis of triethyltin and tributyltin compounds, triethyltin chloride for the tripropyltin compound. In the case of the simultaneous analysis of three trialkyltin compounds, methylmercury chloride was used as internnal standard (Fig. 2A).

Calibration graphs. Standard mixtures containing various amounts (1–100 ng/ml) of trialkyltin chlorides and a approximately equal amount of internal standard in *n*-hexane were prepared. Under the GC conditions shown in Fig. 2, linear calibration graphs were established for peak heights of trialkyltin chlorides, indicating good working ranges for these compounds. Detection limits reached $1 \cdot 10^{-12}$ g for trialkyltin chlorides.

Sample preparation. Trialkyltin compounds in tissues appear to exist predominantly as unmetabolized compounds and detectable levels of hydroxy compounds. These compounds were extracted from tissues as their chlorides by a combination of hydrochloric acid and organic solvents. They could easily be extracted by using weak and non-polar solvents such as benzene, toluene, hexane and ethyl acetate. In the case of the simultaneous extraction of triethyl-, tripropyl- and tributyltin com-

pounds from tissues, however, ethyl acetate was more suitable because trimethyl- and triethyltin are only slightly polar. The recovery from double extractions with ethyl acetate was about 98%.

Further purifications were performed by the addition of *n*-hexane to the concentrated extract, followed by stepwise elution with *n*-hexane–ethyl acetate on a silica gel column. Ethyl acetate-soluble and *n*-hexane-insoluble substances were eliminated by the replacement of the extraction solution with *n*-hexane. Three trialkyltin chlorides were eluted with *n*-hexane–ethyl acetate (4:1) and could be separated from other weakly polar substances which were adsorbed on the silica gel column.

Analysis of standard sample

Two standard mixtures of trialkyltin chlorides were analyzed using the whole procedure. The recoveries at the 10^{-9}-g level were 95–107% and those at the 10^{-7}-g level were 99–100% (Table I).

TABLE I

ANALYSIS OF STANDARD SAMPLE

Two standard mixtures of trialkyltin compounds were analyzed using the whole procedure. An approximately equal amount of Pr_3SnCl was employed as internal standard for the analysis of Et_3SnCl and Bu_3SnCl, and Et_3SnCl for Pr_3SnCl. GC conditions as in Fig. 2B. Each result is the average of five determinations (mean ± standard error).

Standard mixture		*Average*	
Compound	*Added (ng)*	*Found (ng)*	*Recovery (%)*
I			
Et_3SnCl	1.0	1.07 ± 0.05	107.3 ± 5.0
Pr_3SnCl	1.5	1.52 ± 0.01	101.3 ± 1.2
Bu_3SnCl	2.0	1.90 ± 0.03	95.0 ± 2.0
II			
Et_3SnCl	100.0	100.2 ± 0.9	100.2 ± 0.9
Pr_3SnCl	150.0	150.6 ± 1.2	100.4 ± 0.8
Bu_3SnCl	200.0	198.0 ± 0.8	99.0 ± 0.4

Addition studies

The application of the method to the analysis of trialkyltins in mammals was studied by conducting recovery tests on animal tissues. Approximately equal amounts (about 50 pmoles of each) of trialkyltin chlorides were added to various rabbit tissues and the recoveries were determined (Table II and Fig. 3). The average recoveries ragned from 97 to 106%. No difference in recoveries was seen among different organs.

Application to in vivo studies

The GC method was applied to trialkyltin homologues in rats after oral (Figs. 4 and 6) or subcutaneous (Fig. 5) administrations. Fig. 4 shows the time course of the distribution of tributyltin compounds in organs after a single oral administration. The concentration in each organ, with the exception of the serum, reached a maxi-

TABLE II

RECOVERY OF TRIALKYLTIN COMPOUNDS ADDED TO RABBIT TISSUES *IN VITRO*

Three trialkyltins (about 50 pmole of each) were added to various tissues (0.1–1.0 g) and subjected to the GC method. Internal standards as in Table I. GC conditions as in Fig. 2B. Each result is the average of five determinations (mean ± standard error).

Compound	*Added (ng)*	*Organ*	*Average*	
			Found (ng)	*Recovery (%)*
Et_3SnCl	12.0	Blood	12.4 ± 0.3	103.3 ± 2.1
		Liver	11.8 ± 0.2	98.3 ± 2.0
		Kidney	12.5 ± 0.3	104.2 ± 1.6
		Brain	12.0 ± 0.3	100.0 ± 2.3
Pr_3SnCl	14.0	Blood	14.9 ± 0.2	106.4 ± 1.9
		Liver	14.4 ± 0.3	102.9 ± 1.7
		Kidney	14.2 ± 0.3	101.4 ± 2.1
		Brain	13.5 ± 0.2	96.6 ± 1.4
Bu_3SnCl	16.0	Blood	15.9 ± 0.3	99.6 ± 2.0
		Liver	16.1 ± 0.3	100.6 ± 1.8
		Kidney	15.7 ± 0.2	98.1 ± 1.0
		Brain	15.5 ± 0.2	96.9 ± 1.2

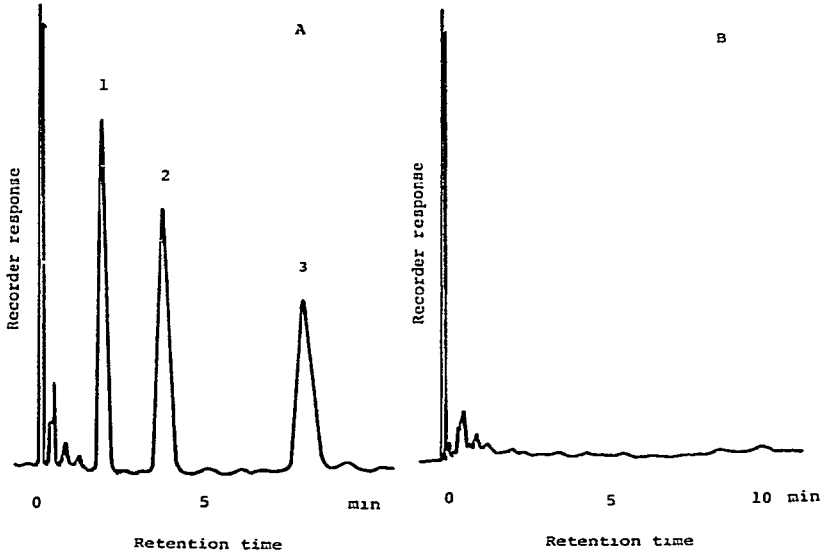

Fig. 3. Gas chromatograms of liver extracts of rabbit treated with trialkyltin compounds (A) and untreated (B). Details as in Fig. 2B.

mum after 24 h. Fig. 6 indicates that the ileum and jejunum are the main absorption sites of trialkyltin compounds in the gut. The results given in Fig. 5 suggest an enterohepatic circulation of trialkyltin compounds.

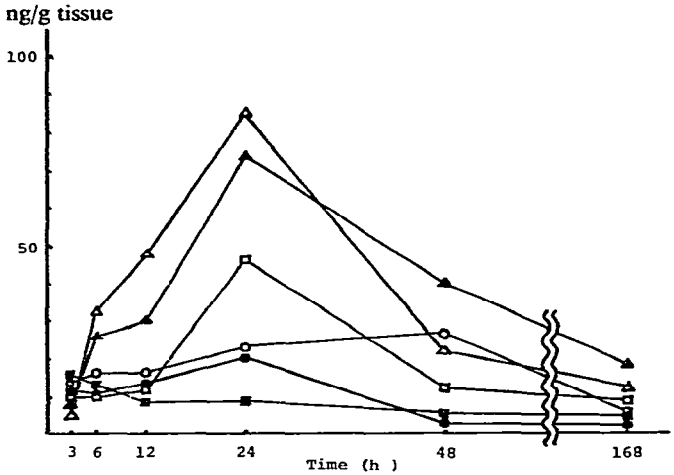

Fig. 4. Distribution patterns of tributyltin compounds in rat organs after a single oral administration. Tissue samples (about 0.1 g) from rats given tributyltin chloride (6.5 mg per kg body weight) were subjected to the GC method using tripropyltin chloride as an internal standard. DC conditions are in Fig. 2A Tributyltin compounds are expressed as chlorides (ng/g of tissue wet wt.). Each point represents the mean of four determinations. ○, Liver; ●, brain; △, kidney; ▲, spleen; □, red cell; ■, serum.

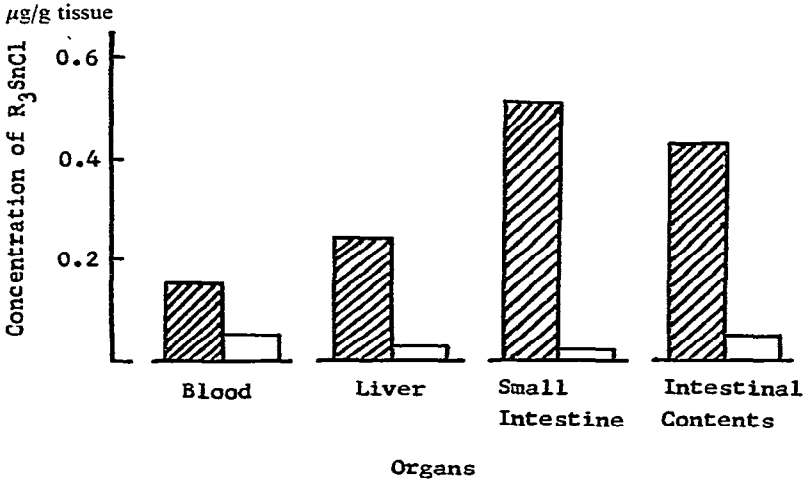

Fig. 5. Distribution of trialkyltin compounds in rat organs after repeated subcutaneous injections. Tissue samples (about 0.1 g) from rats injected subcutaneously with triethyltin chloride and tributyltin chloride (each 10 mg per kg body weight) were subjected to the GC method using tripropyltin chloride as an internal standard. GC conditions as in Fig. 2B. Trialkyltin compounds are expressed as chlorides (μg/g of tissue wet wt.). Each value represents the mean of four determinations. Compounds: ▨, triethyltin; □, tributyltin.

Through these experiments, it was confirmed that the method is applicable and sufficiently accurate for the simultaneous determination of trialkyltin compounds in biomaterials containing more than 1 ng/g of tissue wet weight. The application of this method to studies on the dealkylation of alkyltin compounds in mammals will be published elsewhere.

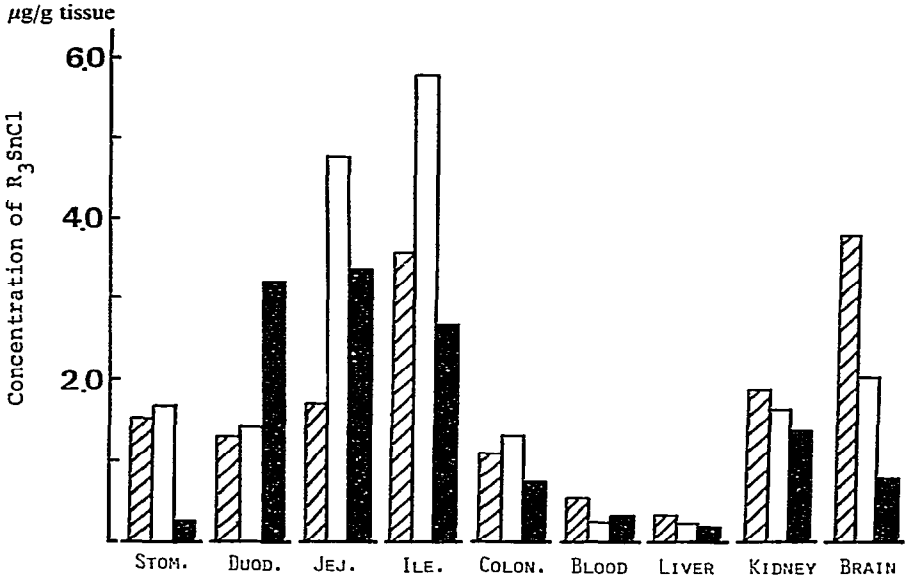

Fig. 6. Distribution of trialkyltin compounds in rat organs after repeated oral administrations. Tissue samples (0.1–1 g) from rats administered orally with a mixture of three trialkyltin chlorides (each 3 mg per kg body weight) five times, once every 12 h, were subjected to the GC method using methylmercury chloride as an internal standard. GC conditions as in Fig. 2A. Trialkyltin compounds are expressed as chlorides (μg/g of tissue wet wt.). Each value represents the mean of four determinations. Compounds: ▨, triethyltin; □, tripropyltin; ■, tributyltin.

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