Determination of Butylin Metabolites in the Mouse Liver by Flameless Atomic Absorption Spectrophotometry

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ABSTRACT. A new analytical method for observation of the metabolic status of butylin compounds in the mouse liver was devised by a combination of extraction, purification and separation followed by quantitative analysis of each butylin compound. After the extraction of all tin compounds from liver homogenate with ethyl acetate, these compounds were purified by combination of the fractional extract with organic solvents and column chromatography. The purified fraction was also analyzed by thin-layer chromatography, identifying each tin compound from differences in mobility on a silica gel plate. The tin content in the each separated spot on the plates was measured by flameless atomic absorption spectrophotometry after extraction by acid treatment. About 90% of tin was recovered by this method from the liver of mice which had been administered tri- or dibutylin compound orally. This method will be useful for quantification of each metabolic product formed from butylin compounds in vivo.—KEY WORDS: butylin compound, metabolism, mouse liver.


The toxicity of organotin compounds has attracted special interest because of the expanding utilization of and pollution with organotin compounds in the form of plastic stabilizers, catalytic agents, industrial and agricultural biocides, anti-fouling paints and pesticides [9, 11]. Among these organotin compounds, tributyltin and dibutyltin compounds have been recognized as particularly important environmental pollutants because of their widespread industrial and agricultural applications [3].

Depending upon the number of organic moieties, butylin compounds are classified as mono-, di-, tri- or tetrabutylin. In general, the toxicity of organotin compounds to mammals decreases from tri- to monoorganotins [3, 9]. Among butylin compounds, tri- and dibutyltin compounds have been shown to cause injury to the bile duct and liver in experimental animals [1, 2].

It was suggested that the metabolism of butylin compounds by cytochrome P450 enzymes may play a role in the induction of biological effects, because tributyltin was found to undergo hydroxylation followed by dealkylation to produce dibutylin, monobutyltin and inorganic tin compounds in the presence of microsomes and NADPH in vitro [4–6, 8]. In addition to the differences in toxicity of butylin compounds dependent upon the number of combined butyl groups, the conversion of butylin compounds by various biological systems makes it difficult to elucidate the real biological effects of each butylin compound in vitro and in vivo. In order to solve this problem, it is necessary to observe the metabolism of butylin compounds in biological materials. The present report therefore aims at devising an analytical method for the observation of the metabolic status of butylin compounds in biological samples.

The tin compounds (tributyltin chloride; TBTC, dibutyltin dichloride; DBTC, monobutyltin trichloride; MBTC, tin chloride; SnCl₂) added to a mouse liver homogenate were extracted and purified by the method of Iwai et al. [7] and Suzuki et al. [10] as shown in Fig. 1. After the homogenized sample was acidified with HCl, added tin compounds were extracted with ethyl acetate. The same extraction procedure was repeated twice. The extract was then concentrated under reduced pressure at room temperature. The concentrated solution was mixed with n-hexane, and the supernatant fraction obtained by centrifugation was concentrated again as above, the final volume being accurately quantified for the measurement of the tin concentration. The n-hexane fraction containing butylin compounds was dried before the purification.
procedure. The butyltin compounds were purified by fractional extraction with n-hexane and acetonitrile, and column chromatography was performed with Florisil (Florisil Co.) and Wakogel C-100 (Wako Pure Chemical Ind.). The final residue was dissolved in diethyl ether (1 ml). For the measurement of the tin contents in these fractions, aliquots of the samples were subjected to flameless atomic absorption spectrophotometry (Shimadzu, Model AA-650 equipped with Model GFA-2 graphite furnace atomizer), as soon as possible under the same instrumental operative conditions as Iwai et al. [7]. As shown in Table 1, 93–97% of the tin compounds added to the liver homogenate were extracted with ethyl acetate, and the final recovery was about 90% of the added tin compounds in nearly all of the tin compounds used in our experiments.

In order to demonstrate the applicability of this method in analyzing the metabolic status of butyltin compounds in vivo, these extraction and purification procedures were also carried out with the liver obtained from mice 3 hr after the oral administration of TBTC. Because 87% of tin compounds extracted from the intact liver were also recoverable by this method, it was found that this extraction procedure is suitable for application to in vivo experiments. In the purification procedure, which had been developed for marine products by Suzuki et al. [10], about 87% of the extracted tin compounds were recovered (Table 1). Consequently, we concluded from these experiments that the extraction and purification procedures mentioned above could be applied to determine the butyltin compounds in biological materials such as a mouse liver.

Next, thin layer chromatography as reported by Kimmel et al. [8] was used for the separation and detection of the butyltin compounds in the purified samples. The butyltin compounds extracted and purified from the liver of mice, which had received oral administration of TBTC 3 hr before the experiment, were separated on a Silica gel 60 TLC plate (Merek) into 6 spots, (Bu₄SnX (TBTC), (HOBu)Bu₂SnX, \(\gamma-C=O-Bu\)Bu₂SnX, Bu₂SnX₂ (DBTC), BuSnX₃ (MBTC) and SnX₄), in the same manner as demonstrated in vitro by Kimmel et al. [8]. These results suggest that, in addition to the typical butyltin compounds such as TBTC, DBTC, MBTC and SnCl₄, at least two intermediate metabolites were detectable by thin layer chromatography in the intact liver obtained from mice administered TBTC. The thin layer chromatography also detected three spots identified as DBTC, MBTC and SnCl₄ in the liver of mice administered DBTC in vivo.

Immediately after the separation of the tin compound spots on the plates, the silica gel spots were collected individually in test tubes, and the butyltin compounds were extracted by boiling for 1 hr in 10% HNO₃ for TBTC, the intermediate metabolites and DBTC, and 3N HCl for MBTC and SnCl₄. After acidification, the samples were centrifuged and the tin content in the supernatant fraction was measured by flameless atomic absorption spectrophotometry under the same conditions mentioned above. One hundred percent of the tin compounds applied to these silica gel plates were recovered from the plates by these two acid treatments, (10% HNO₃ for TBTC and DBTC, and 3N HCl for MBTC and SnCl₄) (Table 2), and the total amount of tin recovered from the silica gel plate was about 90% of tin applied to the plate in the experiments with the samples obtained from the liver of mice administered TBTC or DBTC in vivo (Table 3). However, these acid treatments had to be performed as soon as possible after the procedure of thin layer chromatography, because the recovery of tin from the silica gel plate decreased if the time interval between these procedures was prolonged. It is therefore suggested that these procedures could be satisfactorily applied to the quantitative analysis of various butyltin compounds in the mouse liver.

Because organotin compounds binding with alkyl radicals generally undergo dealkylation by micosomes [4–6, 8], there has been significant interest in investigating the relation between biological effects and the metabolites of organotin compounds. These biological conversions of organotin compounds may be responsible for the confusion of biological effects induced by the different butyltin compounds. Therefore, it is hoped that the precise

### Table 1. Recovery of the tin compounds added to the mouse liver homogenate and the liver of mice administered TBTC in vivo

<table>
<thead>
<tr>
<th>Tin compound added to the homogenate</th>
<th>Intact liver (in vivo treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBTC</td>
<td>DBTC</td>
</tr>
<tr>
<td>Recovery in extraction (%)</td>
<td>96.1±6.8</td>
</tr>
<tr>
<td>Final recovery (%)</td>
<td>91.3±7.4</td>
</tr>
</tbody>
</table>

\(n=4\)

\(a\) The mice were administered orally with TBTC (180 μmol/kg) 3 hr before the experiments. The final recoveries were calculated using the amounts of the extracted tin as 100%. Each value is mean±S.D.

### Table 2. Recovery of butyltin compounds from the silica gel plate after analysis by thin layer chromatography

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBTC</td>
</tr>
<tr>
<td>10% HNO₃, 100°C, 1 hr</td>
<td>100</td>
</tr>
<tr>
<td>3N HCl, 100°C, 1 hr</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Recovery of the butyltin compounds obtained from the liver of mice administered TBTC or DBTC in vivo from the silica gel plate after analysis by thin layer chromatography

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBTC (%)</th>
<th>Intermediates</th>
<th>DBTC (%)</th>
<th>MBTC (%)</th>
<th>SnCl4 (%)</th>
<th>Total tin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBTC (180 μ mol/kg)</td>
<td>1.9±0.8</td>
<td>0.4±0.2</td>
<td>39.6±13.3</td>
<td>34.2±16.0</td>
<td>12.0±6.2</td>
<td>90.9±10.6</td>
</tr>
<tr>
<td>DBTC (60 μ mol/kg)</td>
<td>N.D.†</td>
<td>N.D.†</td>
<td>84.6±7.5</td>
<td>3.8±2.2</td>
<td>1.0±0.5</td>
<td>89.9±8.3</td>
</tr>
</tbody>
</table>

a) Not detected.

The tin compounds in the liver were analyzed at 3 hr after the oral administration of butyltin compounds. Each value is mean±S.D. (n=4)

biological effects of a specific butyltin compound may be deduced from the analysis of the metabolic status of these compounds in biological materials. From the results described above, we may conclude that the metabolic status of butyltin compounds in biological materials can be assessed by our analytical method. This method will be useful in many laboratories because it does not require any special equipment other than a flameless atomic absorption spectrophotometer, which is already widely used in the laboratories studying heavy metals.

REFERENCES