Excretion of Triethyllead, Diethyllead and Inorganic Lead in Rabbits after Injection of Triethyl Neopentoxy Lead

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Abstract: Triethyl neopentoxy lead (TEneoPOL: (C2H5)3Pb [OCH2C(CH3)3] liquid) was administered to rabbits in a single dose of 10 mg/kg body weight (5.4 mg Pb/kg body weight), and the urinary and fecal excretions of lead were measured to determine the fate of this triethyllead derivative. About 4% of the administered amount of was excreted into the urine during the 7 days after the injection; and about 68%, into the feces. In other words, the fecal excretion of total lead was about 17 times as great as the urinary excretion. About 85% of the urinary excretion of total lead was composed of diethyllead (Et2Pb2+), and about 92% of the fecal excretion consisted of inorganic lead (Pb2+). The major chemical species of lead excreted in the urine was Et2Pb2+, while the major species excreted in the feces was Pb2+. These results were similar to those of administration of tetraethyllead (Et4Pb) to rabbits. One of the 7 rabbits died on the day following the injection, and TEneoPOL, a triethyllead derivative, proved to be no less toxic than Et4Pb. However, this compound is immediately hydrolyzed by the ambient moisture to form a white solid compound, so that it is not accompanied by as great a risk of airway exposure as Et4Pb.

Key words: TEneoPOL, Triethyllead, Diethyllead, PZT thin film, MOCVD

Introduction

In the electronic industry, PZT (Pb(Zr, Ti)O3) film, a ferroelectric thin film used as the memory element in the dynamic random access memory (DRAM) chip, is produced by allowing it to grow on a silicon wafer 6-8 inches in diameter. Lead bisdipivaloymethane (Pb(DPM)2), which occurs as a solid at ordinary temperatures, or tetraethyllead (Et4Pb), which occurs as a liquid at ordinary temperatures, is usually used as the lead material to allow the PZT film to grow by chemical vapor deposition (CVD). In recent years, attempts have been made to allow PZT film to grow by metallorganic chemical vapor deposition (MOCVD) using triethyl neopentoxy lead (TEneoPOL), which occurs as a liquid, instead of Et4Pb1). MOCVD uses zirconium tetrateriarybutoxide (Zr(O-t-C4H9)) (a liquid) and titanium tetraisopropoxide (Ti(O-i-C3H7)4) (a liquid) as organic metals in addition to TEneoPOL and O2. It has been ascertained that with TEneoPOL a ferroelectric thin film grows which has better properties than that with Pb(DPM)2).

As is Et4Pb, TEneoPOL ((C2H5)3Pb[OCH2C(CH3)3]) is an alkyllead compound, which occurs as a light yellow, clear, hygroscopic liquid, and was synthesized as a substitute for Et4Pb. Et4Pb, the best known of all tetraalkylleads, was once used extensively and in tremendous quantities as an excellent antiknock agent for gasoline engines, and has often caused highly lethal poisoning2-5). Since Et4Pb is readily metabolized to triethyllead (Et3Pb+) in vivo, Et3Pb+ is considered to be the culprit in Et4Pb poisoning2-5). Since Et4Pb is readily metabolized to triethyllead (Et3Pb+) in vivo, Et3Pb+ is considered to be the culprit in Et4Pb poisoning2-5).

TEneoPOL is one of the aforementioned triethyllead...
derivatives produced for use on a commercial scale in the electronic industry. It is, therefore, necessary for the user to have sufficient knowledge of its toxicity and metabolism. To our knowledge, however, there have been no reports on the biological effects of this compound. In this study, TEneoPOL was administered to rabbits, and the chemical species of metabolized lead excreted into the urine and feces and the behavior of the lead in organs were investigated to study the fate of this triethyllead derivative.

Materials and Methods

Reagents

A sample of TEneoPOL that was manufactured for in-house use by Tri Chemical Lab. Ltd., Japan, was used in the study. The full chemical name for this compound is triethyllead-2,2-dimethylpropoxide (\((\text{C}_2\text{H}_5)_3\text{Pb}[\text{OCH}_2\text{C(CH}_3)_3]\)). It has a molecular weight of 381.52, and the sample had a purity of 99.9999% (as total lead). TEneoPOL has the following physicochemical properties. It occurs as a light yellow, hygroscopic liquid having an alcohol-like odor. It boils at 73°C/0.24 mm Hg, and has a specific gravity of 1.48. It is unstable in water, but stable in dry air. It gradually decomposes at high temperatures (>1000°C), and is inflammable. TEneoPOL needs to be handled in a vacuum container or a container whose air has been replaced with dry argon. On reacting with the moisture in ambient air, it is instantly converted from a liquid to a solid. Assay of the sample of this compound for lead content in our laboratory yielded 98.6% \(\text{Et}_3\text{Pb}^+\), plus 0.5% diethyllead (\(\text{Et}_2\text{Pb}^2+\)), 0.2% inorganic lead (\(\text{Pb}^{2+}\)), and 0.7% \(\text{Et}_4\text{Pb}\) as impurities.

\(\text{Et}_4\text{Pb}\) standard solution: Tetraethyllead (Strem Chemicals Inc., U.S.A.) was washed with a mixture of redistilled water and ether. In 25 ml of benzene, 0.166 g of the washed \(\text{Et}_4\text{Pb}\) was dissolved, and the solution was diluted with ethanol before use.

\(\text{Et}_3\text{Pb}^+\) Standard solution: Triethyllead chloride (Alfa Division, Ventron Corp., U.S.A.) was purified with a water-ethanol mixture. In 100 ml of redistilled water, 3.30 mg of this purified compound was dissolved for use as the stock solution.

\(\text{Et}_2\text{Pb}^2+\) Standard solution: Diethyllead dichloride was synthesized from \(\text{Et}_3\text{Pb}\) via diethyllead dibenzoate by Heap’s method\(^6\), and purified with a water-ethanol mixture. In 100 ml of redistilled water, 3.35 mg of this purified compound was dissolved for use as the stock solution.

Pb\(^{2+}\) Standard solution: This standard solution was prepared by dissolving 100 mg of metal lead (Soekawa Chemical Co., Ltd., Japan) in 100 ml of 1 N \(\text{HNO}_3\).

Of these standard solutions, 3 solutions (\(\text{Et}_3\text{Pb}^+\), \(\text{Et}_2\text{Pb}^2+\) and \(\text{Pb}^{2+}\)) were diluted with redistilled water as necessary before use.

Nitric acid, perchloric acid, ammonium hydroxide, sodium diethylthiocarbamate (SDDC), and methyl isobutyl ketone (MIBK) of reagent grade for toxic metal analysis were used. The other reagents used were of special grade.

Experimental animals

Seven female Japanese white rabbits (designated as A through G), weighing 3.0–3.7 kg, were used. TEneoPOL was administered to the rabbits at a single dose of 10 mg/kg body weight (5.4 mg Pb/kg body weight) by drawing 20–25 \(\mu\)l of undiluted TEneoPOL into a microsyringe (Terumo Co., Japan) and injecting it into the auricular vein of the rabbits. One (G) of the 7 rabbits died the following day.

After the injection, the rabbits were housed in metabolic cages to collect their urine and feces separately, and they were given free access to water and food for 7 days. The day following the injection, 3 (D-F) of the rabbits were sacrificed to determine the chemical species of lead in their organs. The other 3 rabbits (A-C) were sacrificed 7 days after the injection to determine the residual amount of lead in the body.

Procedures for lead determination

To determine of \(\text{Et}_4\text{Pb}\), \(\text{Et}_3\text{Pb}^+\), and \(\text{Et}_2\text{Pb}^2+\) in the urine, urine samples were repeatedly subjected to extraction with organic solvents and to back extraction of the water layer to sequentially separate the target chemical species by MIBK extraction, and the lead species in the extracts were determined by flame atomic absorption spectrometry (A.A.S.)\(^7\). To determine \(\text{Pb}^{2+}\), \(\text{Pb}^{2+}\) was allowed to precipitate by Bolanowska’s method\(^8\) in the urine sample from which \(\text{Et}_4\text{Pb}\) had been removed, and the precipitate was dissolved in nitric acid for A.A.S.

To determine \(\text{Et}_4\text{Pb}\), \(\text{Et}_3\text{Pb}^+\), and \(\text{Et}_2\text{Pb}^2+\) in the feces and organs, redistilled water was added to each feces or organ sample, and the sample was homogenized. The homogenate was treated in a similar manner to the urine samples, and then subjected to A.A.S.

To determine total lead in the urine, feces, and organs,
Each sample was transferred into a Teflon decomposition vessel (Uniseal Decomposition Vessels Ltd., Israel), and after the addition of 5 ml of concentrated nitric acid, was decomposed by heating (130°C, 90 min.), then extracted with MIBK, and subjected to A.A.S. In presenting the total lead measurements as tables and graphs, the sum of Et₄Pb, Et₃Pb⁺, Et₂Pb²⁺, and Pb²⁺ was used instead of the actual measurements to allow the respective species ratios of lead to total 100%.

Results

Urinary and fecal total lead excretion

Table 1 shows the ratios of total lead excreted into the urine and feces during the 7 days after intravenous injection of TEneoPOL at a dose of 10 mg/kg body weight.

The urinary excretion of total lead during the 7 days accounted for about 4% of the administered lead dose, with about 3/10 of this amount excreted 1 day after, and only about 1/20 excreted 7 days after the injection.

The fecal excretion of total lead during the 7 days, on the other hand, amounted to about 68% of the dose of lead administered. Fecal excretion 2 days after the injection accounted for about 1/2 of the 7-day excretion, and fecal excretion 7 days after the injection decreased to about 1/20.

Consequently, fecal excretion of total lead was about 17 times greater than its urinary excretion.

Chemical species of urinary lead

Figure 1 shows variations in the urinary excretion of each lead species during the 7 days.

Chemical species of fecal lead

Figure 2 shows variations in the fecal excretion of each lead species during the 7 days.

Chemical species of lead in organs

Table 2 shows the concentration of each lead species in the organs of rabbits sacrificed 1 day after and 7 days after the injection.

The total lead concentration in the brain of rabbits sacrificed 1 day after the injection consisted of Et₃Pb⁺ alone. The total lead concentration in the liver consisted of about 78% Et₃Pb⁺, about 14% Pb²⁺, and about 8% Et₃Pb⁺. The total lead concentration in the kidneys of the same rabbits

Table 1. Percentages of total lead, Et₃Pb⁺, Et₂Pb²⁺ and Pb²⁺ in urine and feces during 7 days after intravenous injection of triethyl neopentoxy lead (TEneoPOL) at a single dose of 10 mg/kg body weight (3 rabbits)

<table>
<thead>
<tr>
<th>Days after injection</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total lead</td>
<td>Et₃Pb⁺</td>
</tr>
<tr>
<td></td>
<td>(% of dose)</td>
<td>(% of dose)</td>
</tr>
<tr>
<td>1</td>
<td>1.07</td>
<td>0.071</td>
</tr>
<tr>
<td>2</td>
<td>1.03</td>
<td>0.097</td>
</tr>
<tr>
<td>3</td>
<td>0.56</td>
<td>0.039</td>
</tr>
<tr>
<td>4</td>
<td>0.30</td>
<td>0.020</td>
</tr>
<tr>
<td>5</td>
<td>0.22</td>
<td>0.024</td>
</tr>
<tr>
<td>6</td>
<td>0.17</td>
<td>0.018</td>
</tr>
<tr>
<td>7</td>
<td>0.16</td>
<td>0.012</td>
</tr>
<tr>
<td>Before injection</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>7-day excretion</td>
<td>3.51</td>
<td>0.281</td>
</tr>
</tbody>
</table>

*: Total lead = tetraethyllead + triethyllead + diethyllead +inorganic lead. % mean value (n=3).
was composed of about 72% $\text{Et}_3\text{Pb}^+$, about 19% $\text{Pb}^{2+}$, and about 9% $\text{Et}_2\text{Pb}^{2+}$, and the total lead concentration in the blood comprised about 38% $\text{Pb}^{2+}$, about 34% $\text{Et}_3\text{Pb}^+$, and about 28% $\text{Et}_2\text{Pb}^{2+}$. The total lead concentration in the B bile of these rabbits was made up of about 89% $\text{Et}_2\text{Pb}^{2+}$, about 9% $\text{Pb}^{2+}$, and about 2% $\text{Et}_3\text{Pb}^+$, and the total lead concentration of the cecal concentrations consisted of about 79% $\text{Pb}^{2+}$, about 13% $\text{Et}_2\text{Pb}^{2+}$, about 8% $\text{Et}_3\text{Pb}^+$, and a trace of $\text{Et}_4\text{Pb}$.

In other words, the $\text{Et}_3\text{Pb}^+$ concentration decreased in the following order: brain, liver, kidneys, blood, $\text{Et}_2\text{Pb}^{2+}$ was excreted in large amounts into the bile and urine. $\text{Pb}^{2+}$ accounted for the largest part of total lead in the cecal concentrations.

These ratios of lead species in the organs did not greatly change 7 days after the injection, but only $\text{Pb}^{2+}$ was detected in the blood and cecal concentrations (Fig. 3).
Discussion

Today, hardly any tetraalkyllead-containing gasoline is used in Japan. However, Et₄Pb used to be used in large quantities as an antiknock agent in gasoline throughout the world. In recent years, attention has been called to its use as an electronic material, and this compound has found a new industrial application. However, Et₄Pb is a deadly poison, and because it occurs as a very volatile liquid, handling it is not easy. This has necessitated the production of an ethyllead compound that occurs as a less volatile liquid at room temperature, and TEneoPOL was produced as such a less volatile compound. This compound, very unstable in water, is immediately hydrolyzed by the moisture in air into a white solid form, which is probably triethyllead hydroxide (Et₃PbOH).

We set the test dose of TEneoPOL for administration to rabbits at 10 mg/kg body weight (5.4 mg Pb/kg body weight). This amount corresponds to 2/3 of its LD₅₀, 15 mg/kg body weight (8.1 mg Pb/kg body weight), found in a toxicity study of the compound by Yamauchi et al.⁹ using percutaneous administration of single doses in mice. In this study, one of the 7 rabbits died on the day following the injection. In a previous study⁹, an aqueous solution of triethyllead chloride was administered intravenously to one rabbit at a dose of 5.0 mg Pb/kg body weight, and the rabbit died. In that instance, the rabbit squeaked because of distress during the intravenous injection of triethyllead chloride. After the injection, the animal became weak, lay on its side, and died a few hours later. The injection of TEneoPOL, 5.4 mg Pb/kg body weight, which contained a similar amount of lead, caused less distress to the rabbit than the injection of triethyllead chloride. The two organic lead compounds differ in the stress they cause to rabbits.

When lead excretion by the rabbits administered Et₄Pb⁹ was compared with excretion by the TEneoPOL-administered rabbits, the 7-day urinary excretion of total lead accounted for about 4% of the administered dose of lead in each instance. The 7-day fecal excretion of total lead amounted to about 56%, which was close to the 68% in this study. The excretions by chemical species showed that the urinary excretion of total lead consisted of about 68% Et₂Pb⁺, compared with about 85% in this study, while the fecal excretion of inorganic lead accounted for about 91% of total lead, which was similar to the about 92% in this study.

The Et₃Pb⁺ ratio to the total lead in the organs was similar for both the Et₄Pb- and TEneoPOL-dosed rabbits; and the Et₂Pb₂⁺ ratio in the B bile was 93%, which was close to the 89% in this study. Consequently, the administration of Et₄Pb and that of TEneoPOL produced similar organ levels and excretion of each chemical species of lead. The lead dose of Et₄Pb administered in this study was 1.4 times that of TEneoPOL.

It has been ascertained that the major chemical species of lead that is excreted in humans and rabbits exhibiting Et₄Pb toxicity is Et₃Pb⁺ in the urine¹⁰-¹⁶. It is also known that a large amount of lead is excreted in the stools of humans with Et₄Pb poisoning¹⁷. Similar results were achieved in the experiment in which Et₄Pb was administered to rabbits, with the fecal excretion of total lead being about 14 times greater than the urinary excretion, and Pb²⁺ accounting for about 85% of the fecal excretion of total lead 2 days after the injection¹⁰. The high fecal excretion of Pb²⁺ is due to the fact that the lead is excreted as Et₂Pb₂⁺ into the bile is dealkylated in the intestinal tract¹⁰.

The Et₃Pb⁺ produced in the metabolism of Et₄Pb is thought to be the culprit in Et₄Pb poisoning, rather than Et₄Pb itself being toxic². Buck and Kumuro¹⁰ reported that when equal amounts of Et₄Pb and Et₃Pb⁺ were injected into rats, they showed similar reactions to both compounds that were entirely different from the signs of Et₄Pb²⁺ or Pb²⁺ toxicity.

Bischoff et al.²¹ observed that following administration of Et₄Pb and Et₃Pb⁺ to rabbits, the animals showed similar toxic signs in response to both compounds. Macle¹⁷ reported that the frogs, pigeons, rats, rabbits, dogs, and cats that he used in his experiments all showed similar general
pharmacological reactions to Et₄Pb.

Yamauchi et al.9 observed that when mice and hamsters were subcutaneously injected with TEneoPOL, they exhibited anxiety and mild tremors of their extremities, suggestive of the characteristic CNS damage of Et₄Pb poisoning, and then discovered that with reference to its LD₅₀ of 15 mg/kg body weight of mice, these compounds are toxicologically similar to Et₄Pb.

TEneoPOL is not currently listed as a lead species in the ordinance on prevention of tetraalkyllead poisoning under the Industrial Safety and Health Law of Japan. However, since it is as toxic as Et₄Pb, particular caution needs to be exercised in its handling. Because this compound is converted from a liquid to a solid on hydrolysis by ambient moisture, there is little risk of exposure to its vapor, but it is necessary in handling this compound to wear a gas mask, a face guard and protective gloves and work on it in a draft chamber. In this instance, monitoring the humidity at the working site is a must. It is because dry air increases the risk of exposure to TEneoPOL vapor.

For this reason, the risk of exposure to its vapor may be considered not as great as that of Et₄Pb vapor, except at work sites maintained at extremely low humidity.

Acknowledgment

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References