Metabolic Fate of cis- and trans-Chlordane in Mice

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Introduction

Chlordane was once used in large quantities for termite control in Japan, but a law prohibiting their manufacture, import and use was implemented in September 1986. However, chlordanes (cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor and oxychlordane) are extremely stable chemically and only slowly decompose: their half lives in the environment are estimated to be 15~20 years or more1). Similar to known organochlorine compounds such as DDT, dieldrin and PCB, chlordanes also contaminate fishes, shellfishes and various other foods through the food chain in the ecological system2).

In order to elucidate the behavior of chlordanes taken into the body via foods, we studied the tissue distribution and clearance of cis-chlordane and trans-chlordane, which are major components of technical chlordane, in mice. The results are described below.

Methods

1. Animals and treatment

Four-week-old male ICR strain mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals) were purchased and reared for three weeks prior to the test in a room maintained at 24±1°C, 50±10% humidity, and a 12-hr light/dark cycle. Those aged 7 weeks, which weighed about 34 g were used for the test. During the experiment, animals were given food (pellets: Oriental Yeast Co.) and water ad libitum.

The dose levels of two congeners were based on the data that the oral LD50 value was 430 mg/kg for technical chlordane in mice3). A 7.8 ml solution obtained by dissolving 53.1 mg of cis-chlordane and 53.0 mg of trans-chlordane in salad oil (6.8 mg/ml for each of the congeners) was used for the test. The 0.1 ml solution was orally administered to mice using a stomach tube (20 mg/kg each of the congeners). On days 1, 2 and 4 and in weeks 1, 2, 4, 8, 12, 20, 28 and 52 after dosing, blood samples were collected from the femoral artery in six animals. After exsanguination by decapitation, liver, kidney, spleen, muscle, brain and mesenteric adipose (after the 8th week) were excised and rinsed in cold normal saline. These were used as the samples for analysis.

2. Reagents

Cis-chlordane and trans-chlordane were purchased from Wako Pure Chemical Co., and oxychlordane was supplied by Velsicol Pacific Limited. For other reagents, products from Wako Pure Chemical Co. of pesticide-residue-analysis grade or reagent grade were used.

3. Analytical procedure

Analysis was performed according to the method of analysis for chlordanes specified in Official Methods of Analysis of The Association of Official Analytical Chemists4).

i) Extraction

The sample was stirred in 40 ml of acetone for 1 min at 24,000 r.p.m. using an ULTRA-TURRAX homogenizer (Janke & Kunkel Co.). To the resultant mixture was further added 60 ml of hexane, and it was stirred under the same conditions to extract chlordanes. The residue was re-extracted with 50 ml of hexane. The extracts were combined, washed with 70 ml of 5% NaCl solution, dehydrated with anhydrous Na2SO4, and concentrated to about 10 ml under reduced pressure on a water bath set at 40°C.
or below using a rotary evaporator.

**ii) Acetonitrile partitioning**

As for the adipose tissue and liver, the concentrate obtained using procedure i) was transferred to a 100 ml separatory funnel, after which hexane was added to make the total volume 15 ml. It was then extracted three times by shaking with 30 ml of acetonitrile saturated with hexane. The acetonitrile extracts were combined and washed with 20 ml of hexane saturated with acetonitrile. The washing liquid was washed with 30 ml of acetonitrile saturated with hexane. The washings and the extracts were combined and concentrated to about 0.5 ml under reduced pressure. The concentrate was dissolved in 10 ml of hexane.

**iii) Florisil cleanup**

A 20-mm i. d. column was filled with 20 g of Florisil-PR (which had been heated at 130°C for at least 16 hours and then cooled in a desiccator) using the wet method with hexane, and about 8 g of anhydrous Na₂SO₄ was further superimposed on this column. The concentrates obtained through procedures i) and ii) were charged in the above column, and chlordanes were eluted with 200 ml of hexane containing 6% ether. The eluate was concentrated to about 10 ml under reduced pressure at 40°C or below using a rotary evaporator, and further concentrated to 1 ml in a stream of nitrogen. This was used as the test solution.

**iv) Apparatus**

A Hitachi M-80 was used for MS analysis and a Hitachi M-003 was used for the data system.

**v) Conditions for GC-MS (SIM) measurement**

- Liquid stationary phase, 2% OV-1: support, Uniport HPS 100–120 mesh; column, glass column (length 1.5m, i. d. 3mm); temperature, 255°C for injection, 240°C for the column and 265°C for the separator; ionization voltage, 20 eV. Ions measured by SIM (m/z) were 375 for cis-chlordane, 375 for trans-chlordane and 389 for oxychlordane.

### Results and Discussion

**1. Tissue distribution of the administered congeners**

The concentrations of cis- and trans-chlordanes in the tissues reached the maximum on day 1 after dosing (Fig. 1). The tissue concentration of cis-chlordane on day 1 was highest in muscle (1260±377 ppb), and its order was followed by liver (377±88 ppb), kidney (136±29 ppb), brain (56±15 ppb), spleen (36±13 ppb) and blood (29±8 ppb). Cis-chlordane disappeared quickly from the tissues, the concentrations on day 4 after dosing became 1/83 (muscle), 1/8 (liver), 1/17 (kidney), 1/8 (brain) and 1/7 (blood) of the values on day 1. The cis-chlordane in the spleen disappeared completely by day 4 after dosing. Complete elimination of the congener was observed on day 7 in blood, on day 14 in muscle, kidney and brain, and on day 28 in the liver.

The metabolic fate of trans-chlordane in the tissues was also similar to that of cis-chlordane (Fig. 1). The tissue concentration of trans-chlordane on day 1 was highest in muscle (766±233 ppb), and its order was followed by liver (103±29 ppb), kidney (82±17 ppb), brain (37±10 ppb), spleen (34±14 ppb) and blood (22±6 ppb). Trans-chlordane also disappeared quickly from various tissues, and its concentration on day 4 decreased to 1/56 (muscle), 1/6 (liver), 1/12 (kidney), 1/5 (brain) and 1/5 (blood) of the corresponding concentrations on day 1. Trans-chlordane in the spleen disappeared totally by day 4 after dosing, and it was not detectable in the blood on day 7 or in muscle, liver, kidney and brain on day 14.

Of the in vivo behaviors of cis- and trans-chlordanes, the data concerning excretion have been described in detail. However, data on the tissue accumulations of these congeners have seldom been reported. Therefore, the half lives of their concentrations in the tissues were investigated in the present study.

The rate of decrease of cis- and trans-chlordane in various tissues was rapid, and no difference in the half life in the tissues was observed between the two (Table 1). The half life of cis-chlordane was shortest in the spleen (0.6 day) and longest in the brain (1.9 days). The values for other tissues ranged
Fig. 1 Time courses of the concentrations of cis-chlordane (○) and trans-chlordane (●) in mouse tissues after a single oral administration. (a) muscle; (b) liver; (c) kidney; (d) spleen; (e) brain; (f) blood
Each point shows the mean ± S.D. (ng/g tissue) of 6 mice.

from 1 to 1.5 days. As for trans-chlordane, its half life was shortest in the spleen (0.5 day) and longest in the liver (2 days). The values for other tissues ranged from 1 to 1.5 days. Thus the half life of the congeners was different between tissues, while the values for the two congeners obtained from each tissue were similar.

Barnett et al.\(^8\) reported that when a single dose of cis-/trans-chlordane (3:1) labelled with \(^{14}\)C was orally administered to rats, 70% of cis-chlordane and 60% of trans-chlordane were excreted by 24 hours after dosing, and more than 90% of both congeners were excreted by day 7. The results of the
present study conducted at a higher dose agreed well with those of their report. As several studies have heretofore reported\(^6\)\(^-\)\(^9\), cis-chlordane is more easily excreted than trans-chlordane, but accumulated cis-chlordane is metabolized more slowly than trans-chlordane. This tendency was found with higher concentrations of cis-chlordane in various tissues and was particularly prominent in the liver.

The two congeners (cis- and trans-chlordane) had completely disappeared from all the tissues examined by week 4 after dosing despite the single administration of the comparatively high dose (20 mg/kg each). On the other hand, Takeda et al\(^{10}\) reported that the two congeners were detected only in trace amounts in the adipose tissue even after two-weeks repeated administrations of a technical chlordane to rats. Therefore, in humans, the fact that the two congeners are detectable in vital samples such as mother’s milk suggests a continuous exposure to these congeners.

2. Tissue distribution of metabolites

Cis-chlordane and trans-chlordane were quickly metabolized to oxychlordane after dosing, and that metabolite had the highest concentration in the liver on day 2 after dosing and in other tissues on day 1 (Fig. 2). The concentration of oxychlordane in the liver was particularly high (1918\(\pm\)410 ppb), followed by muscle (569\(\pm\)186 ppb), kidney (326\(\pm\)55 ppb), brain (226\(\pm\)44 ppb), spleen (126\(\pm\)27 ppb) and blood (103\(\pm\)22 ppb). Oxychlordane accumulated in the tissues at concentrations several times higher than those of cis- and trans-chlordane except in muscle, even if it was in due consideration of difference in molecular weight between the administered congeners (M. W. 410) and their metabolite (M. W. 424). Trans-chlordane is known to be metabolized to oxychlordane more easily than cis-chlordane is. Loss of oxychlordane from the tissues was extremely slow (Table 2), and it remained in various tissues at 10% (muscle)\(\sim\)35% (blood) of the concentrations on day 1 (day 2 for the liver) after dosing even in week 4, when the administered congeners had disappeared from all the tissues. The ratio of accumulation was 7.6% (muscle)\(\sim\)17.5% (blood) of the maximum concentration after dosing, in week 8 after dosing: 6.0% (muscle)\(\sim\)10.2% (brain) in week 20; and 1.0% (blood)\(\sim\)5.1% (muscle) in week 52 (year 1). On the other hand, no splenic concentration of the metabolite was detected in week 8 or later. In the adipose tissue examined from week 8 after dosing, the concentration was 2890\(\pm\)337 ppb in week 8, 1911\(\pm\)331 ppb in week 20 and 648\(\pm\)306 ppb in week 52, concentrations that were much higher than in other tissues. As shown by the arrow (\(\uparrow\)) in Fig. 2, the regression curves for oxychlordane were diphasic at week 4 for the muscle, week 8 for the liver and brain, and week 12 for the kidney and blood. The half-life values obtained from these curves are shown in Table 3. The half life of oxychlordane was calculated to be 10 days (muscle)\(\sim\)25 days (kidney, blood) before week 8 after dosing. Its value was prolonged to 83 days (blood)\(\sim\)417 days (muscle) thereafter.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Half life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>cis-Chlordane</td>
</tr>
<tr>
<td>Liver</td>
<td>0.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.6</td>
</tr>
<tr>
<td>Brain</td>
<td>1.9</td>
</tr>
<tr>
<td>Blood</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 1 Half lives of two congeners in mouse tissues after a single oral administration of a cis- and trans-chlordane mixture

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weeks after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle a)</td>
<td>10.2</td>
</tr>
<tr>
<td>Liver b)</td>
<td>16.9</td>
</tr>
<tr>
<td>Kidney a)</td>
<td>27.0</td>
</tr>
<tr>
<td>Spleen a)</td>
<td>18.3</td>
</tr>
<tr>
<td>Brain a)</td>
<td>27.0</td>
</tr>
<tr>
<td>Blood a)</td>
<td>35.0</td>
</tr>
</tbody>
</table>

a) The concentration on day 1.
b) The concentration on day 2.
c) Not detected.

Table 2 Relative distribution of oxychlordane found in mouse tissues after a single oral administration of a cis- and trans-chlordane mixture (%)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>First (days)</td>
</tr>
<tr>
<td>Liver</td>
<td>20 132</td>
</tr>
<tr>
<td>Kidney</td>
<td>25 190</td>
</tr>
<tr>
<td>Spleen</td>
<td>15</td>
</tr>
<tr>
<td>Brain</td>
<td>20 146</td>
</tr>
<tr>
<td>Blood</td>
<td>25 83</td>
</tr>
</tbody>
</table>

Table 3 Half life of oxychlordane in mouse tissues after a single oral administration of a cis- and trans-chlordane mixture
Fig. 2 Time courses of the concentrations of oxychlordane in mouse tissues after a single oral administration of a cis- and trans-chlordane mixture. (a) muscle; (b) liver; (c) kidney; (d) spleen; (e) brain; (f) blood; (g) adipose
Each point shows the mean ± S.D. (ng/g tissue) of 6 mice.

Conclusion

When cis-chlordane and trans-chlordane, major constituents of the technical chlordane used for termite control, were orally administered to mice, they tended to be concentrated in muscle in the body. Clearance from the body was comparatively rapid, and its half life was estimated to be 2 days for liver and brain. It was indicated that cis-chlordane accumulates in several tissues with higher concentrations than trans-chlordane. Both of the congeners were rapidly metabolized in the body to form oxychlordane. This metabolite was liable to accumulate in the liver, and its elimination rate from various tissues was extremely slow. Although the half life of oxychlordane was more or less dependent on the tissues, its
value was around 20 days up to week 8 after dosing. However, the subsequent elimination rate was extremely slackened and the half life was prolonged by 4~7 times (83~155 days) in most tissues and by 42 times (417 days) in the exceptional case of muscle.

Several reports have been published on in vivo behaviors of cis-, trans-chlordane and their metabolite, oxychlordane. However, the details concerning their regression curves and half life in various tissues are unknown because of insufficient analytical data. The present study revealed the diphasic character (oxychlordane) of the regression curve for each tissue and the half life for each phase, and elucidated the detailed in vivo distribution and concentration changes of cis-, trans-chlordane and their metabolite, oxychlordane.

Based on the above data, it is assumed that cis-chlordane and trans-chlordane ingested via fishes and meats by humans are quickly metabolized to oxychlordane, which accumulates in the adipose tissue and other tissues over a prolonged period of time. Since oxychlordane, being highly accumulative,
is more toxic than cis-chlordane or trans-chlordane\textsuperscript{11}, it is necessary to study in more detail the in vivo behavior of oxychlordane, for instance at lower doses.

Summary

A single dose of a cis-chlordane and trans-chlordane mixture (1:1) was orally administered to mice (total dose: 40 mg/kg), and the metabolic fate of the two congeners administered and their major metabolite, oxychlordane, in various tissues, was studied from day 1 after dosing to week 52. cis-chlordane and trans-chlordane showed the highest concentrations on day 1 after dosing, and disappeared on day 14 except in the liver. The half life in the tissues was approximately one day for both congeners. On the other hand, oxychlordane was observed in various tissues from day 1 after dosing, reaching the maximum concentration on day 1 or 2 showing considerably higher concentrations than those of the congeners. The rate of decrease of oxychlordane in the tissues was extremely slow compared to the congeners. It was found that oxychlordane remained in the tissues even in week 52 (year 1) after dosing. The regression curve for the tissue oxychlordane concentration was diphasic after or at around week 8 after dosing; the half life was approximately 20 days in the first phase and was prolonged to over 100 days in the second phase. These results suggest that the cis-chlordane and trans-chlordane taken into the human body via foods disappear rapidly from the tissues but that oxychlordane, their metabolite, remains in the body over a prolonged period of time.

References


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マウスにおけるシス-トランス-クロルデンの体内動態

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マウスにシス-クロルデン，トランス-クロルデンの混合物（1：1）を1回経口投与（total 40mg/kg）し，投与後1日目から52週目に渡って，投与した2種の化合物および主代謝産物オキシクロルデンの各組織への分布および蓄積性について検討した。シス-クロルデン，トランス-クロルデンは投与後1日目に各組織で最高値を示し，肝臓を除いて14日目には各組織から消失していた。これら2種の化合物の各組織中濃度半減期は概ね1日であった。一方，主代謝産物のオキシクロルデンは投与後1日目より各組織で認められ，投与後1日目あるいは2日目に最高値を示し，投与した2種の化合物よりかなり高い濃度で存在していた。各組織でのオキシクロルデンの消失速度は投与した2種の化合物に比べて極めて遅く，投与後52週目でも各組織に存在していた。各組織でオキシクロルデンの消失曲線は投与後8週目付近を境に2相性を示した。各組織中濃度半減期は第1相では概ね20日前後であったが，第2相では半減期が100日以上に延長していた。これらの結果，ヒトが食品を経由して取り込んだシス-クロルデン，トランス-クロルデンも速やかに組織から消失し，代謝産物オキシクロルデンの形で長期に渡り生体内に存在することが示唆された。

Key words：Chlordane, Oxychlordane, Mouse, Distribution, Excretion
クロルデン，オキシクロルデン，マウス，分布，排泄