Metabolism of 1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane, and 1-Chloro-2,2-bis(p-chlorophenyl)ethene in the Hamster

Barry Gold and Galen Brunk

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

ABSTRACT

The urinary metabolites of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), and 1-chloro-2,2-bis(p-chlorophenyl)ethene in female hamsters are reported. The principal metabolite of both DDT and DDD is 2,2-bis(p-chlorophenyl) acetic acid. DDT- and DDD-treated animals also excreted small amounts of DDD, 1-chloro-2,2-bis(p-chlorophenyl) acetaldehyde, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene, 2-hydroxy-2,2-bis(p-chlorophenyl)acetic acid, and 2,2-bis(p-chlorophenyl)ethanol. One-Chloro-2,2-bis(p-chlorophenyl) acetaldehyde is metabolized to afford significant amounts of 2,2-bis(p-chlorophenyl)acetic acid, 2,2-bis(p-chlorophenyl)ethanol, 2-hydroxy-2,2-bis(p-chlorophenyl) acetic acid, 2,2-bis(p-chlorophenyl)acetaldehyde, and 1,1-bis(p-chlorophenyl) ethan-1,2-diol. These results indicate that the metabolic disposition of DDT in the hamster, a species refractory to DDT tumorigenesis, is very similar to that observed previously in the mouse, a species sensitive to DDT tumorigenicity. The one exception is that the hamster is not nearly as efficient as the mouse in converting DDT to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene, a metabolite that is tumorigenic in both species.

INTRODUCTION

We have previously investigated the metabolism of DDT, DDD, and DDMU in the mouse and demonstrated that metabolism of DDT affords small quantities of DDMU epoxide, as evidenced by excretion of aOH-DDA in mouse urine (10, 11). This epoxide was shown to be mutagenic in the Ames system without enzymic activation (10). It was also suggested that DDA, a metabolite of covalent adducts of DNA, account for the tumorigenicity of DDA-CI, along with DDMU epoxide, as reported previously (13). The [3H]DDT was diluted with DDD to give a final specific activity of 0.134 mCi/mmol, and the [3H]DDT was diluted with DDD to give a specific activity of 2.4 mCi/mmol. DDA, DDOH, DDE, and M-nitro-N-mercapto-3-hydroxy-3-p-chlorophenylpropan-1-one were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Other compounds, 1-1-txs(p-chlorophenyl)ethene, 1-chloro-2,2-bis(p-chlorophenyl)ethene, DCHO, methyl 2-hydroxy-2,2-bis(p-chlorophenyl)acetate, methyl 2,2-bis(p-chlorophenyl)acetate, and DDMU-diol, were prepared by methods reported previously (11).

Animal Treatment. Female Syrian golden hamsters were used for metabolism studies. The animals weighed approximately 130 g and were from the Eppley Institute colony. The DDT (100 mg/kg containing 124.7 μCi of [3H]DDT), DDD (500 mg/kg containing 23.5 μCi of [3H]DDD), and DDMU (500 mg/kg) were dissolved in 0.4 ml of olive oil and administered by gavage. For each compound, 3 pairs of hamsters were kept in glass metabolism cages (Crown Scientific, Oriand Park, Ill.) that allowed separate collection of urine and feces. Collections were made at 24-hr intervals, with a total collection time of 72 hr. The collectors on the metabolism cages were submerged in solid CO2 to freeze samples as they were excreted. During the study, the hamsters were removed from the cages for 2 hr every 24 hr and given access to food. Water was provided ad libitum.

Metabolite Analysis. Samples from DDT- and DDD-treated hamsters were examined by reverse- and normal-phase HPLC, as described previously (10). Samples from DDMU-treated hamsters were analyzed by gas-liquid chromatography (11).

RESULTS

Urinary metabolite levels for DDT, DDD, and DDMU were quantitated for each of the three 24-hr collection periods (Tables 1 to 3). DDA and, presumably, aOH-DDA are excreted as conjugates (3, 9, 17, 24) and, accordingly, their quantitation involved base hydrolysis of the urine followed by acidification to yield the free acids, which were then extracted into diethyl ether and...
derivatized with excess diazomethane to afford methyl 2,2-bis(p-chlorophenyl)acetate and methyl 2-hydroxy-2,2-bis(p-chlorophenyl)acetate. The values of DDA, αOH-DDA (quantitated as their methyl esters) and DDOH are corrected for recovery (11).

The major urinary metabolite of DDT is DDA, which accounts for 56% of the excreted radioactivity, with excretion peaking during the second 24-hr interval of collection. The other polar metabolites, αOH-DDA and DDOH, were found in small amounts, and excretion of the former rose throughout the collection periods, in contrast to excretion of DDOH, which is maximum in the first 24-hr period. DDD, DDE, DDMU, and unchanged DDT are also detected, and their excretion rate remained relatively steady during the 72 hr of collection.

Urinary metabolites derived from DDD are similar to those found from DDT. DDA is by far the major metabolite, with its rate of excretion decreasing with time. DDOH and αOH-DDA are also detected, with no clear difference in their rate of excretion over the collection period. Small amounts of DDE and DDMU were again detected, in addition to unmetabolized DDD.

The profile of DDMU metabolites differed qualitatively and quantitatively from that observed with the DDT and DDD. DDA is still the major metabolite, but its level is only approximately 2-fold higher than that of DDOH, in contrast to the approximately 60- and approximately 300-fold difference observed with DDT and DDD, respectively. Excretion of αOH-DDA is also significantly increased. In addition, DDOH and DDDU-diol, 2 metabolites not seen in the DDT and DDD studies, are observed in large amounts. The excretion rate for all the metabolites, with the exception of αOH-DDA, clearly decreases after the first 24-hr collection. The only nonpolar compound detected is unchanged DDMU, and it is only seen in the first collection period.

**DISCUSSION**

The 2 major stable, fat-soluble metabolites of DDT are DDE and DDD (3, 9, 16, 24). The incidence of liver tumors relative to control animals is increased by DDT (14, 21, 22) and DDE in mice (22) and by DDD to a moderate degree in male mice only (22). However, DDD markedly increases the lung tumor incidence in both sexes (21). The tumorigenicity of DDT is at most marginal in the rat (4, 7, 8, 18, 20), and the hamster is reportedly refractory to DDT tumorigenesis (1, 5, 12). To determine if this species difference is related to differences in the metabolic disposition of DDT, we conducted a comparative study in the mouse and hamster.

The formation of DDA, the major urinary metabolite of DDT, was previously believed to involve a sequential route via DDD, DDMU, 1-chloro-2,2-bis(p-chlorophenyl)ethane, 1,1-bis(p-chlorophenyl)ethylene, DDOH, and DDCHO intermediates (Chart 1) (3, 6, 15-17). Recently, it has been suggested that the metabolic data for the production of DDA in the mouse are better reconciled with a mechanism of direct hydroxylation of DDD on the 1-ethane carbon to initially afford DDA-CI, which can either directly hydrolyze to DDA or acylate cellular nucleophiles (10) (Chart 2). The basis for this pathway in the mouse is the approximately 1:2

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**Table 1**

**Urinary metabolites of DDT in female Syrian hamsters**

<table>
<thead>
<tr>
<th>Metabolites (µg)</th>
<th>Collection period (hr)</th>
<th>Unchanged DDT</th>
<th>DDE</th>
<th>DDD</th>
<th>DDMU</th>
<th>DDA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>αOH-DDA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>DDOH&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14.4 ± 10.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>754.2 ± 241.5</td>
<td>8.6 ± 3.6</td>
<td>25.2 ± 11.7</td>
</tr>
<tr>
<td>0-24</td>
<td></td>
<td>13.3 ± 11.0</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>1400.1 ± 227.1</td>
<td>22.6 ± 11.1</td>
<td>8.5 ± 2.1</td>
</tr>
<tr>
<td>48-72</td>
<td></td>
<td>5.5 ± 5.0</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>390.6 ± 36.7</td>
<td>28± 0.4</td>
<td>6.5 ± 5.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>33.2 ± 19.9</td>
<td>0.8 ± 0.1</td>
<td>2.3 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>2544.9 ± 274.8</td>
<td>59.8 ± 11.8</td>
<td>40.2 ± 15.4</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>95</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Corrected for recovery (11).
<sup>b</sup> Derivatized to the corresponding methyl ester with excess diazomethane and quantitated as the methyl ester.
<sup>c</sup> Mean ± S.D.
<sup>d</sup> Limit of detection, <0.01 µg.
ratio of DDA to DDOH in DDMU-treated animals versus the approximately 100:1 and 1200:1 ratio in DDT- and DDD-treated animals, respectively (Table 4). In addition, no DDCHO or DDNU-diol is seen with DDT and DDD, in contrast to their detection upon administration of DDMU (11). The large ratio of DDA to DDOH in DDT- and DDD-treated animals (Tables 1 and 2) and the failure to detect DDCHO or DDNU-diol confirm that the pathway in Chart 2 is also operative in the hamster.

Mouse studies have also revealed that DDMU, a primary metabolite of DDE and DDD, is epoxidized to afford DDMU epoxide, which results in urinary excretion of αOH-DDA (Chart 3) (11). This α-chloroepoxide was synthesized and was demonstrated to be mutagenic in the Ames system without enzyme activation (11). Detection of αOH-DDA in hamster urine after administration of DDT, DDD, and DDMU (Tables 1 to 3) establishes that a similar and as efficient pathway exists in the hamster.

It is not possible to rigorously compare the quantitative differences in the metabolism of DDT, DDD, and DDMU between the mouse and hamster, because different absolute doses were applied in the 2 studies (Table 4). The same doses in mg/kg of body weight were used, but sequestering of the administered compounds in adipose tissue and metabolite excretion are not necessarily linear with dose/kg (2). In previous mouse studies,
we have observed that increasing the dose of DDD by a factor of 2 does not result in a doubling of the level of metabolites excreted (10). Still, the oxidative metabolic pathways for the disposition of DDT, DDE, and DDMU to DDA in the mouse and hamster appear closely related. Therefore, it is difficult to rationalize that formation of DDMU epoxide or DDA-C1, compounds capable of covalently binding to DNA, can account for species-specific tumorigenicity of DDT in the mouse. It is, of course, possible that species differences may arise from differences in repair of DNA lesions and other postbinding events.

There is, however, a previously reported species difference in the metabolism of DDT which is also apparent in our studies and involves conversion of DDT to DDE. The latter compound being a proximate tumorigenic form of DDT in the hamster (19). The role of DDE in the tumorigenicity of DDT in the mouse is presently under investigation.

REFERENCES

Metabolism of 1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane, and 1-Chloro-2,2-bis(p-chlorophenyl)ethene in the Hamster

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