**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)ethene, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene, 2-hydroxy-2,2-bis(p-chlorophenyl)acetic acid, 2,2-bis(p-chlorophenyl)ethanol. 1-Chloro-2,2-bis(p-chlorophenyl)ethene 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 tumorigenicity, 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, the major urinary metabolite of DDT, does not arise from a sequential metabolic pathway, as proposed previously (3, 6, 15-17) (Chart 1) but rather is produced by hydroxylation of the chlorinated side chain of DDD (Chart 2) (11). A hydroxylation of this type would initially afford DDA-CI. This acid chloride can hydrolyze to DDA or react with other nucleophilic molecules. DDA-CI, along with DDMU epoxide, could, via the formation of covalent adducts of DNA, account for the tumorigenicity in the mouse (14, 22, 23). The hamster, in contrast to the mouse, is refractory to DDT tumorigenesis (1, 5, 12); therefore, the metabolism of DDT in the hamster was studied to determine if a difference in metabolism, such as the production of DDMU epoxide or DDA-CI, could account for the species difference exhibited by DDT.

**MATERIALS AND METHODS**

**Chemicals.** Radioactive [phenyl-1-14C]DDT (specific activity, 1.80 mCi/mmol) and [phenyl-3H]DDT (specific activity, 4.65 mCi/mmol) were purchased from California Bionuclear Corp. (Sun Valley, Calif.) and New England Nuclear (Boston, Mass.), respectively. The DDMU was prepared as reported previously (13). The [3H]DDT was purified by preparative HPLC (column, 22.1-mm x 25-cm Zorbax ODS (DuPont Instruments, Wilmington, Del.); solvent, 10% H2O-90% CH3OH; flow rate, 10 ml/min; detection, 238 nm) and shown to be pure by analytical HPLC (column, 4.6-mm x 25-cm Ultrasphere ODS 5μ (Altex, Berkeley, Calif.); solvent, 9% H2O-91% methanol; flow rate, 1.0 ml/min; detection, 238 nm). 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 DDT to give a specific activity of 2.4 mc/mmol. DDA, DDD, DDE, and N-methyl-N'-nitro-N-nitrosoguanidine were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Other compounds, 1,1-bis(p-chlorophenyl)ethene, 1-chloro-2,2-bis(p-chlorophenyl)ethene, DDCHO, 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 150 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, Orland Park, III.) 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...
derivated 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. DDE, 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, DDDO 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) but 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)ethene, DDHOH, and DDDU 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-Cl, 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

| Table 1
<table>
<thead>
<tr>
<th>Metabolites of DDT in female Syrian hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three pairs of hamsters (approximately 130 g) were given DDT (100 mg/kg containing 124.7 μCi of [phenyl-3H]DDT) by gavage in olive oil (400 μl). Urine collections were made at 24-hr intervals, with a total collection time of 72 hr. The urine was heated with base and acidified, and metabolites were extracted into diethyl ether for separation by HPLC and quantification by liquid scintillation counting.</td>
</tr>
<tr>
<td>Metabolites (μg)</td>
</tr>
<tr>
<td>Collection period (hr)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>0-24</td>
</tr>
<tr>
<td>24-48</td>
</tr>
<tr>
<td>48-72</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>* Corrected for recovery (11).</td>
</tr>
<tr>
<td>* Derivatized to the corresponding methyl ester with excess diazomethane and quantitated as the methyl ester.</td>
</tr>
<tr>
<td>* Mean ± S.D.</td>
</tr>
<tr>
<td>* Limit of detection, &lt;0.01 μg.</td>
</tr>
</tbody>
</table>
Table 2

Urinary metabolites of DDD in female Syrian hamsters

Three pairs of hamsters (approximately 130 g) were given DDD (500 mg/kg containing 23.5 μCi of [phenyl-
ul-14C]-DDD) by gavage in olive oil (400 μl). Urine collections were made at 24-hr intervals, with a total
collection time of 72 hr. The urine was heated with base and acidified, and metabolites were extracted into
diethy ether for separation by HPLC and quantitation by liquid scintillation counting.

<table>
<thead>
<tr>
<th>Collection period (hr)</th>
<th>Unchanged</th>
<th>DDE</th>
<th>DDMU</th>
<th>DDA, b</th>
<th>αOH-DDA, b</th>
<th>DDOH, a</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>8.4 ± 5.9</td>
<td>0.0</td>
<td>0.0</td>
<td>11468.5 ± 1246.8</td>
<td>63.4 ± 24.4</td>
<td>33.5 ± 8.5</td>
</tr>
<tr>
<td>24–48</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>7538.6 ± 2567.7</td>
<td>69.9 ± 10.4</td>
<td>12.2 ± 2.6</td>
</tr>
<tr>
<td>48–72</td>
<td>1.6 ± 0.4</td>
<td>0.0</td>
<td>0.0</td>
<td>1504.3 ± 415.2</td>
<td>42.0 ± 21.2</td>
<td>20.2 ± 10.5</td>
</tr>
<tr>
<td>Total</td>
<td>10.0 ± 5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>20511.0 ± 3775.6</td>
<td>175.2 ± 55.5</td>
<td>66.0 ± 11.4</td>
</tr>
</tbody>
</table>

%<1

* Corrected for recovery (11).
* Derivatized to the corresponding methyl ester with excess diazomethane and quantitated as the methyl
ester.
* Mean ± S.D.
* Limit of detection, <0.03 μg.

Table 3

Urinary metabolites of DDMU in female Syrian hamsters

Three pairs of hamsters (approximately 130 g) were given DDMU (500 mg/kg) by gavage in olive oil (400 μl). Urine was
collected at 24-hr intervals with a total collection time of 72 hr. The urine was heated with base and acidified, and
metabolites were extracted into diethyle ether for separation and quantitation by gas-liquid chromatography and integration
of peak areas.

<table>
<thead>
<tr>
<th>Collection period (hr)</th>
<th>Unchanged</th>
<th>DDA, b</th>
<th>αOH-DDA, b</th>
<th>DDOH, a</th>
<th>DDCHO</th>
<th>DDNU-diol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>26.5 ± 7.7</td>
<td>2253.7 ± 419.0</td>
<td>569.4 ± 92.7</td>
<td>1574.5 ± 254.9</td>
<td>414.8 ± 55.2</td>
<td>1179.2 ± 224.4</td>
</tr>
<tr>
<td>24–48</td>
<td>0.0</td>
<td>973.8 ± 153.7</td>
<td>644.5 ± 125.1</td>
<td>197.8 ± 53.0</td>
<td>137.0 ± 22.3</td>
<td>0.0</td>
</tr>
<tr>
<td>48–72</td>
<td>0.0</td>
<td>401.4 ± 31.9</td>
<td>295.6 ± 41.3</td>
<td>62.0 ± 18.6</td>
<td>35.0 ± 31.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>26.5 ± 7.7</td>
<td>3629.0 ± 534.7</td>
<td>1509.5 ± 158.5</td>
<td>1836.1 ± 215.4</td>
<td>587.6 ± 64.8</td>
<td>1179.2 ± 222.4</td>
</tr>
</tbody>
</table>
%<1                     | 41        | 17      | 21         | 7        | 13     |

* Corrected for recovery (11).
* Derivatized to the corresponding methyl ester with excess diazomethane and quantitated as the methyl
ester.
* Mean ± S.D.
* Limit of detection, <0.05 μg.

Table 4

Comparison of urinary metabolites of DDT, DDD, and DDMU in Swiss mice (10, 11) and Syrian hamsters

<table>
<thead>
<tr>
<th>Administered compound</th>
<th>Species</th>
<th>DDT</th>
<th>DDE</th>
<th>DDD</th>
<th>DDMU</th>
<th>DDA, d</th>
<th>αOH-DDA, d</th>
<th>DDOH, a</th>
<th>DDCHO</th>
<th>DDNU-diol</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT (100 mg/kg)</td>
<td>Mouse</td>
<td>0.1</td>
<td>9.2</td>
<td>2.5</td>
<td>0.6</td>
<td>77.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hamster</td>
<td>33.2</td>
<td>0.8</td>
<td>2.3</td>
<td>0.4</td>
<td>2544.0</td>
<td>59.8</td>
<td>40.2</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>DDD (500 mg/kg)</td>
<td>Mouse</td>
<td>3.7</td>
<td>48.3</td>
<td>3.3</td>
<td>3.3</td>
<td>1265.5</td>
<td>10.2</td>
<td>1.0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hamster</td>
<td>0.5</td>
<td>10.0</td>
<td>0.9</td>
<td>0.9</td>
<td>20511.0</td>
<td>175.2</td>
<td>66.0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>DDMU (500 mg/kg)</td>
<td>Mouse</td>
<td>33</td>
<td>241</td>
<td>185</td>
<td>449</td>
<td>57</td>
<td>135</td>
<td>58</td>
<td>1179</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hamster</td>
<td>27</td>
<td>3629</td>
<td>1510</td>
<td>1836</td>
<td>588</td>
<td>1179</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on at least 3 pairs of animals and representing the total from a 72-hr collection.
* Female.
* Corrected for recovery (11).
* Derivatized to and quantitated as the corresponding methyl ester reported as the free acid.

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 demon-
strated 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) estab-
lishes that a similar and as efficient pathway exists in the
hamster.

It is not possible to rigorously compare the quantitative differ-
ences 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, DDD, 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-CI, compounds capable of covalently binding to DNA, can account for species differences in the metabolism of DDT which is also apparent in our studies and repair of DNA lesions and other postbinding events. It is, of course, possible that species differences may arise from differences in the disposition of DDT, ODD, and DDMU to DDA in the mouse and hamster exposed to DDT. ODD, and DDMU to DDA in the mouse and hamster. Agrie. Food Chem., 22: 904-908, 1974.

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

Barry Gold and Galen Brunk