Carcinogenicity Study with Technical-Grade Dichlorodiphenyltrichloro-ethane and 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene in Hamsters

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ABSTRACT

Studies conducted by others have revealed that 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a proximal metabolite of dichlorodiphenyltrichloroethane (DDT), is a strong hepatocellular carcinogen in mice. Since hamsters appear to be resistant to tumor induction by DDT, we wanted to investigate whether DDE has any neoplastic effect in this species. DDE (99% pure) was mixed into the diet at doses of 500 or 1000 ppm and given to groups of male and female Syrian golden hamsters for life. Another group of animals received a diet containing 1000 ppm technical-grade DDT, and a further group served as control. Groups contained a minimum of 40 hamsters per sex. The tested compounds had no effect on the incidence of tumors at all sites, compared to controls. A specific finding in animals exposed to DDE was the appearance of hepatocellular tumors late in life. They were classified as neoplastic nodules, and the incidence was 15% in females and 47% in males of the 500-ppm DDE dose groups and 21% in females and 33% in males of the 1000-ppm DDE dose groups. None of the untreated or DDT-treated animals had these tumors. Eight animals treated with 1000 ppm DDE and four of those treated with DDT had hyperplastic foci of the liver. In addition, adenocortical adenosomas, spontaneous to Syrian golden hamsters, were more frequent in DDE- and DDT-treated animals than in control animals. These results showing that DDE, but not its parental compound, induces liver cell tumors in hamsters emphasize the importance of this metabolite as a proximal carcinogen of DDT.

INTRODUCTION

On long-term exposure, DDT, a synthetic chlorinated hydrocarbon with powerful pesticidal activity, is carcinogenic to mice (10, 12, 22, 26) and to a lesser extent to rats (3, 5, 20). The susceptibility of common laboratory animals to the carcinogenic effects of this pesticide varies; hamsters, for example, have proved to be resistant to tumor induction by prolonged treatment with DDT (1, 2, 7). In an attempt to explain these different outcomes, metabolic studies were conducted in mice and hamsters. After ingestion of diets containing DDT, one of its proximal metabolites, DDE, was detected at much higher concentrations in organs and tissues of mice than in those of hamsters (6). Although one recent study has provided no evidence of carcinogenicity of DDT and DDE in rats (16), it is conceivable that, at least in certain mammalian species or strains, the metabolic activation to DDE represents a critical step towards tumor induction by DDT. This hypothesis has now received some support for experiments indicating that in mice DDE is more carcinogenic than DDT in that it induces a higher percentage of hepatocellular carcinomas with a shorter latency (16, 23). To date, however, no experiments have been published on the effects of DDE in hamsters, a species in which, for the reasons outlined above, more convincing evidence could be obtained on the role played by certain metabolic derivatives in the production of tumors by DDT.

In the present study, we describe the results of an experiment showing that DDE, but not DDT, is carcinogenic to hamsters. Our finding, together with previous observations made in mice, clearly demonstrates that DDE is a proximal carcinogen of DDT.

MATERIALS AND METHODS

Chemicals. Technical-grade DDT (Geigy, Milan, Italy), of the same batch as used in a previous experiment (20), had an average composition of 70 to 75% p,p'-DDT, 20% o,p'-DDT, and 0.2 to 4% p,p'-TDE. p,p'-DDE, 99% pure, was kindly provided by Ugine Kuhlmann Laboratories (Jarrie, France). The compounds were dissolved in 3% olive oil and mixed into the diet. Pelleted diets were prepared by Piccioni (Brescia, Italy). A periodic evaluation of the DDT and DDE content of the pellets was carried out with the collaboration of the Istituto di Igiene e Profilassi of Genova (Genova, Italy), and values of +10% to -5% of the nominal concentration were found. Repeated gas chromatographic analysis revealed no aflatoxin in either control or experimental diets.

Animals and Procedure. Four- to 5-week-old male and female Syrian golden hamsters were purchased from Charles River Laboratories (Wilmington, Mass.). They were housed in groups of 4 to 5 in Makrolon plastic cages and maintained under standard laboratory conditions. At 8 weeks of age, the animals were divided into 4 groups with an equal number of animals with light, average, and heavy body weight per group: the first group of 45 females and 47 males received 1000 ppm DDE; the second group of 43 females and 40 males received 500 ppm DDE; the third group of 48 animals of each sex were treated with 1000 ppm DDT. A fourth group of 46 females and 45 males served as controls and received the standard diet with added 3% olive oil.

Food and water were given ad libitum. The animals were treated up to 128 weeks of age, at which time the few remaining survivors were killed. Body weight, food consumption, and data on the health of the hamsters were recorded at biweekly intervals thereafter. Animals that were found dead or that were killed because they were moribund were submitted to detailed autopsy. All organs were examined macroscopically and preserved in...
10% buffered formalin. Histological studies were carried out routinely on the liver (one or more section per lobe), spleen, kidneys, adrenal glands, urinary bladder, thyroid, lungs, testes, ovaries, and any other organ that showed gross pathological changes. Nonpathological samples of other organs, including stomach, intestine, salivary and Harderian glands, brain, pituitary, heart, thymus, and lymph nodes, were also taken from same animals at random. The sections were stained with hematoxylin and eosin. A few animals of each group were excluded from the final evaluation because of excessive decomposition or cannibalism. The results on the survival of the animals and on the incidence of tumors were statistically analyzed according to the method of Peto (19).

RESULTS

Toxicity. The survival rates of female and male hamsters given DDT or DDE and of the control group are shown in Chart 1. No significant trends in mortality were observed when the log rank test was applied to these curves. On average, however, treated animals lived longer than did controls, and there was a better survival rate among males than among females, especially late in life (100 weeks or more). There was no immediate explanation to account for these delayed effects, except that amyloidosis of liver, kidney, and adrenals was frequently observed in control animals where 90% of females and 64% of males appeared to be affected by the disease. This compares with 24% females per group among treated hamsters, irrespective of the compound or the dose administered. Depression of amyloidosis was also a common finding in rats exposed to DDT (2), thus suggesting that in laboratory animals DDT and its derivatives have specific inhibitory action on this pathological alteration. A sex-dependent effect of DDT and DDE in mice and rats has been reported recently (16).

Throughout the experiment, treated animals exhibited a delay in mean body weight gain, as compared with controls (Chart 2). This effect was dose related in DDE-treated groups: male animals given in the high dose began to lose weight as early as Week 10; and females given the high dose began by Week 40. Since food consumption was comparable among all groups, including controls, the reduced weight gain may have been due to liver necrosis which affected the animals given 1000 ppm DDE more severely than those given 500 ppm DDE. The effects of DDT on body weight were intermediate between those of the low-dose DDE group and those of the high-dose DDE group.

According to several reports, convulsions and tremors are early signs of intoxication in laboratory animals given DDT or DDE (16, 20). In our study, however, in which doses of comparable toxicity were administered, none of the hamsters showed these reactions, a further indication that this species is highly resistant to acute and chronic toxic effects of DDT and DDE.

Tumor Incidence. The incidence, sites, and types of tumors
Effective no. of animals

<table>
<thead>
<tr>
<th>Tumor-bearing animals</th>
<th>Total no. of tumors</th>
<th>No. of animals with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>DDE, 500 ppm</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>DDE, 1000 ppm</td>
<td>39</td>
<td>16</td>
</tr>
<tr>
<td>DDT, 1000 ppm</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>DDE, 500 ppm</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>DDE, 1000 ppm</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>DDT, 1000 ppm</td>
<td>35</td>
<td>15</td>
</tr>
</tbody>
</table>

- Number of survivors at time of first tumor observed by sex (females, 28 weeks; males, 55 weeks).
- Including liver cell tumors and cavernous hemangiomas.
- One parathyroid adenoma, one ovarian granulosa cell tumor.
- One s.c. fibrosarcoma, one osteosarcoma, one Harderian gland adenoma, one intestinal adenocarcinoma.
- Two parathyroid adenomas, one pituitary adenoma.
- One s.c. fibrosarcoma, one uterine adenocarcinoma, one ovarian granulosa cell tumor.
- One seminal vesicular adenoma, one urinary bladder papilloma.
- One ear region neurinoma, one abdominal malignant tumor not classified.

**Liver cell lesions induced in female and male hamsters untreated or exposed to DDE or DDT**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver cell tumors</th>
<th>Av. no. of nodules/animal</th>
<th>Av. size (mm) of nodules</th>
<th>Wk to first observed tumor</th>
<th>No. of animals with hyperplastic foci only</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/31</td>
<td>(0)</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>DDE, 500 ppm</td>
<td>4/26</td>
<td>(15.4)</td>
<td>0.048</td>
<td>2.0 (1-4)</td>
<td>94</td>
</tr>
<tr>
<td>DDE, 1000 ppm</td>
<td>5/24</td>
<td>(20.8)</td>
<td>0.010</td>
<td>3.2 (1-6)</td>
<td>76</td>
</tr>
<tr>
<td>DDT, 1000 ppm</td>
<td>0/26</td>
<td>(0)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/10</td>
<td>(0)</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>DDE, 500 ppm</td>
<td>7/15</td>
<td>(46.7)</td>
<td>0.017</td>
<td>5.3 (2-18)</td>
<td>105</td>
</tr>
<tr>
<td>DDE, 1000 ppm</td>
<td>8/24</td>
<td>(33.3)</td>
<td>0.013</td>
<td>3.4 (1-10)</td>
<td>105</td>
</tr>
<tr>
<td>DDT, 1000 ppm</td>
<td>0/17</td>
<td>(0)</td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

- Number of animals with liver cell tumors/survivors at time the first liver cell tumor was observed by sex.
- Numbers in parentheses, percentage.
- Numbers in parentheses, range.

Liver Tumors. Tumors of the liver were found in DDE-treated hamsters of both sexes and in one DDT-treated female, but not in female or male controls or in DDT-treated males. The DDT-treated female and another female dosed with 1000 ppm DDE had cavernous hemangiomas. The percentage of animals with liver cell tumors was essentially the same in both DDE-treated groups, with a marginal excess observed in males compared with females (15 and 21% of females and 47 and 33% of males in the low- and high-dose DDE groups, respectively) (Table 2). Other parameters, including the average number of nodules per animal and the size of the nodules, appeared to be slightly increased in females with increased dose, while the opposite was seen in males. The first liver cell tumor was found in a high-dose-DDE female that died at age 76 weeks; the average ages at death of hamsters with liver cell tumors were 104 and 101 weeks in females and 114 and 119 weeks in males given the low and high doses of DDE, respectively.

The liver cell tumors were detected at death as single or multiple nonconfluent, brownish-red or grayish nodules on the liver surface; they sometimes had an hemorrhagic appearance. The nodules ranged from 1 to 16 mm in diameter. Histologically, they were composed of hepatocytes that were larger...
than normal with occasional mitotic figures and a certain degree of polymorphism; in one instance, nodules with small uniform round cells were also observed. The normal lobular architecture was destroyed, and, in general, the cells had a trabecular or more irregular pattern. The nodular cells compressed the surrounding parenchyma and were devoid of hemosiderin, an iron-containing pigment derived from blood cells that usually accumulates in hepatocytes of aging hamsters as a consequence of liver congestion (Fig. 1). In a few cases, the nodules exhibited extensive cystic change or were composed of a mixture of intermediate (mixed clear and basophilic) and clear cells.

No metastases of these tumors were found in lung or any other organ or tissue examined. Based on general criteria already adopted for liver cell tumors of the rat (11), the hepatocellular tumors that we have observed in the present study were classified as neoplastic nodules.

Foci of hyperplastic cells were found in the liver of 8 animals given 1000 ppm DDE and of 4 animals given DDT, but not in animals fed 500 ppm DDE or in controls. These lesions had the approximate dimensions of an hepatic lobule and were composed of hepatocytes with basophilic or sometimes acidophilic cytoplasm. In addition to the focal or diffuse necrosis and amyloidosis already mentioned, other nontumoral diseases of the liver included fatty and cystic degeneration observed in aging animals of all experimental groups including controls.

Adrenal Tumors. Tumors of the adrenal glands were mainly adenomas of the cortex and ranged from microscopic size to 2 to 3 mm in diameter. The other tumors included 2 pheochromocytomas, in one control and in one male given 500 ppm DDE, and 3 cortical carcinomas, in one male given 1000 ppm DDE and in 2 females at each dose of DDE. The incidence of adrenal tumors was high, although not statistically significant, in females of all treated groups (in 5, 18, 21, and 28% of control, low-dose and high-dose DDE- and DDT-treated females, respectively) (Table 1). There was a dose-related effect of DDE on the frequency of adrenal tumors in males, and the frequency of these tumors among DDT-treated hamsters of both sexes closely approached that observed with the high dose of DDE. The average ages at death of the animals with adrenal tumors were 95 weeks in controls and 105 weeks or above in treated animals.

**DISCUSSION**

The purpose of this investigation was to study the effects of lifelong treatment of hamsters with diets containing technical-grade DDT or p,p’-DDE, a major metabolite of p,p’-DDT in mice and rats (18, 24) and in humans (9, 13, 15). Lifelong exposure of hamsters to 1000 ppm DDT did not result in an increase of tumors above the control level, except perhaps for the adrenal neoplasms that occurred in 14% of control and 34% of treated animals. In confirmation of previous experiments in which doses of DDT up to 1000 ppm were used (1, 3, 7), no liver cell tumors were found in our hamsters given DDT, and only 4 had hyperplastic foci of the liver. Somewhat different results were obtained with DDE. When hamsters were treated with 500 or 1000 ppm DDE in the diet, in fact, liver cell tumors were found late in life. On the basis of size and other histological parameters, including loss of lobular architecture and compression of the surrounding normal parenchyma, we have classified these lesions as neoplastic nodules. They were grossly and histologically similar to the liver neoplasms, also termed neoplastic nodules, induced in rats by DDT or phenobarbital (20) and are widely recognized as one stage in the development of hepatocellular carcinomas (4, 21, 27). The absence of these tumors in our controls is in keeping with previous reports that the spontaneous incidence of liver cell tumors in several hamster colonies is nil or extremely low (8, 25). We therefore conclude tentatively that DDT requires metabolic activation to induce tumors and that DDE is a proximal carcinogen of DDT in experimental animals. At least in hamsters, this conclusion is now substantiated by the finding that DDE, but not DDT, induces liver cell tumors. Concerning the long latency (on average more than 100 weeks) required by the neoplastic nodules to become manifest in our experiment, it is well known that promoting agents need prolonged application in order to exert their effects on the target tissues. While there exists no certainty that in hamsters DDE had a promoting-like activity, studies with the model of initiation and promotion have revealed that DDT, the parental compound of DDE, is a promoter of liver carcinogenesis (17).

The effect of DDE on the incidence of liver cell tumors was not dose related, and while the latency to first tumor detected at death was slightly shorter in females than in males (76 and 105 weeks, respectively), a relatively higher percentage of males had these neoplasms (39% males versus 18% females). The observed differences in susceptibility of hamsters to induction of liver cell tumors could not be explained on the basis of our experimental approach, but a sex-linked hormonal effect exerted on the target tissues. While there exists no certainty that in hamsters DDE had a promoting-like activity, studies with the model of initiation and promotion have revealed that DDT, the parental compound of DDE, is a promoter of liver carcinogenesis (17).

As with DDT, an increase was noted in the incidence of adrenocortical adenomas among DDE-treated compared with control animals. This finding, in conjunction with another report that the incidence of same type of tumor was slightly increased in hamsters given DDT (3), indicates a possible carcinogenic effect of the pesticide on adrenal glands of this species. In our study, however, adrenal tumors appeared in old animals (average age, 95 weeks or more), and it cannot be excluded that the incidence in controls, which had a shorter life span, would have been higher with advancing age. For this reason, the observed differences between treated and control animals remain of uncertain meaning, and further investigations are necessary in order to resolve this controversial finding.

In conclusion, the reports that DDE is carcinogenic in mice and our results showing a positive correlation between DDE intake and liver cell tumors in hamsters strongly suggest that this metabolite plays a major role in DDT carcinogenesis. The observation is important in view of the report of exceptionally high accumulation of DDE in the adipose tissues of human populations exposed to diets contaminated with DDT (15).
Further information on the metabolic processes involved in in vivo DDT carcinogenesis could be provided by determining whether p,p'-TDE, another major metabolite of DDT shown to induce tumors in mice and rats (16, 23), is carcinogenic to hamsters.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Histological appearance of representative neoplastic nodules induced in hamsters by lifelong treatment with DDE. A, male treated with 1000 ppm DDE. The specimen show a classical nodule (right) with weakly eosinophilic cytoplasm and compression of adjacent liver cells. B, male treated with 500 ppm DDE. There is no significant penetration of hemosiderin inside the neoplastic cells, and blood cells are accumulated around the external border of the nodule. H & E, × 10.
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