Effect of Mixed-Function Oxidase Modifiers on Metabolism and Toxicity of the Oncogen Dioxane

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ABSTRACT

With both nonradioactive and ¹⁴C-labeled dioxane, the effect of typical inducers and inhibitors of hepatic mixed-function oxidases on the excretion of p-dioxane-2-one, the major urinary metabolite of dioxane, was studied. Pretreatment of rats with the inducers phenobarbital (PB), polychlorinated biphenyls (PCB), and, to a much lesser extent, 3-methylcholanthrene (MC) increased the metabolite excretion and shortened the time of onset of peak excretion of the metabolite. On the other hand, an inhibitor or repressor of mixed-function oxidases such as 2,4-dichloro-6-phenylphenoxyethylamine and cobaltous chloride decreased the metabolite excretion. The results suggest the involvement of mixed-function oxidases in the in vivo metabolism of dioxane. The relationship between the metabolism and the acute toxicity of dioxane was explored. p-Dioxane-2-one [50% lethal dose = 0.79 ± 0.15 g/kg] was considerably more toxic than was dioxane, suggesting that increased metabolism of the latter may bring about increased toxicity. The 50% lethal dose of dioxane in control, PCB-, MC-, and PB-pretreated rats was 5.3 ± 0.1, 4.4 ± 0.1, 5.2 ± 0.1, and 5.4 ± 0.2 g/kg, respectively. Thus, there was an apparent correlation between the metabolism and toxicity of dioxane in PCB- or MC-pretreated rats, whereas PB had no effect on the toxicity of the solvent. The apparent lack of PB effect may be explained by the possibility that p-dioxane-2-one may be further metabolized to exert its toxic effect. Indeed, pretreatment of rats with PCB or MC further increased the toxicity of p-dioxane-2-one, whereas PB appeared to have an opposite effect. It is proposed that the generation of the toxic substance from dioxane may involve a multistep mechanism with p-dioxane-2-one as an intermediate.

INTRODUCTION

p-Dioxane is a commonly used industrial and laboratory solvent; its annual production in the U. S. alone exceeded 7.4 million kg in 1973 (27). The fact that dioxane is also hepatotoxic and nephrotoxic (5, 11, 18) is, however, not well known. Previous investigations from our laboratory (3, 4) have established dioxane as a weak to moderately active hepatocarcinogen. This finding has been confirmed (19). Recently, a World Health Organization study group has evaluated the carcinogenic risk of the solvent (16), and the National Institute for Occupational Safety and Health has proposed to reduce the workplace exposure limit for dioxane from the currently allowed limit of 100 ppm (based on a 30-min sampling time) to 1 ppm, the lowest detectable level (8).

The mechanism of the toxic and carcinogenic action of dioxane is not known. Recent studies from our laboratory (30, 31) and that of Braun and Young (6) have independently identified p-dioxane-2-one (I) and 2- or β-hydroxyethoxyacetic acid (II), respectively, as the major urinary metabolite of dioxane. These 2 compounds are, however, readily interconvertible depending on the pH of the solution and can therefore be considered as identical. It is believed that the acid does not exist as such except in the form of a salt (15); “the tendency to form lactone is so marked that the majority of the acids of this type are known only in the form of the lactone” (24).

It is well documented that hepatic microsomal MFO’s³ are involved in the metabolism of foreign chemicals; the activity of MFO’s can be modulated by various inducers, inhibitors, and repressors (9, 12, 20). For investigation of the role of MFO’s in the metabolism of dioxane, the effect of several such agents on the excretion of p-dioxane-2-one has been studied. In addition, the effect of various inducers on the acute toxicity of dioxane and p-dioxane-2-one has been examined in an effort to elucidate the relationship between the metabolism and toxicity of the solvent.

MATERIALS AND METHODS

p-Dioxane was obtained from Eastman Kodak Co., Rochester, N. Y.; it was purified by fractional distillation over metallic sodium and stored under nitrogen. Uniformly labeled [¹⁴C]dioxane (specific activity, 2.18 mCi/mmol) was custom synthesized by New England Nuclear, Boston, Mass.; the radiochemical purity was greater than 99%. The labeled dioxane was diluted with nonradioactive carrier (20% dioxane in 0.9% NaCl solution) for metabolism stud-

³ The abbreviations used are: MFO, mixed-function oxidase; DPEA, 2,4-dichloro-6-phenylphenoxyethylamine; PB, phenobarbital; PCB, polychlorinated biphenyls (Aroclor 1254); MC, 3-methylcholanthrene; GC, gas chromatography; LDM, 50% lethal dose.

1 Supported by Research Grant CA-15111 from the National Cancer Institute and Grant 922M from The Council for Tobacco Research (U. S. A.). Presented in part at the 68th Annual Meeting of the American Association for Cancer Research, Inc., Denver, 1977 (33).

Received November 15, 1977; accepted March 9, 1978.
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p-Dioxane-2-one, also known as 2-hydroxyethoxyacetic acid 8-lactone, was obtained (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in a polymeric form (white solid), which can be depolymerized by distillation to yield the pure monomer (colorless liquid). The monomer was immediately dissolved in 0.9% NaCl solution and used within 24 hr. DPEA was a gift from Eli Lilly and Co., Indianapolis, Ind. (courtesy of Dr. R. E. McMahon).

Male Sprague-Dawley rats (Holtzman Co., Madison, Wis.) were used throughout the study. They were fed Purina laboratory chow and water ad libitum. In experiments involving pretreatment of rats with inducers of MFO's, 3 types of inducers (namely, PB, PCB, and MC) were used; they were obtained from American Pharmaceutical Co., New York, N. Y.; Monsanto Chemical Co., St. Louis, Mo.; and Eastman Kodak, respectively. PB was dissolved in 0.9% NaCl solution and administered i.p. at a dose of 80 mg/kg body weight daily for 4 consecutive days prior to dioxane administration. MC and PCB were dissolved in corn oil and given i.p., MC at a dose of 40 mg/kg 24 hr prior to dioxane administration and PCB 4 days prior to dioxane administration. The single PCB dose of 500 mg/kg has been routinely used in our laboratory during the last 5 years for induction studies in rats and mice. The rodents given this PCB dose showed no sign of acute toxic effect; the only visible change was enlargement of the liver. Control rats received equivalent amounts of 0.9% NaCl solution or corn oil. For repression of the synthesis of cytochrome P-450, cobaltous chloride (60 mg/kg) was injected s.c. 24 hr prior to dioxane administration according to the method of Tephly and Hibbeln (26). DPEA was dissolved in 0.9% NaCl solution and given (15.9 mg/kg) i.p. 0.5 hr before dioxane administration and 8, 16, and 24 hr thereafter.

For in vivo metabolism studies, rats (95 to 130 g) were housed in stainless steel metabolism cages, 2/cage. Two to 5 pairs of rats were used to determine the effect of each MFO modifier. Dioxane (3 g/kg) was administered i.p., and urine samples were collected at 8- or 12-hr intervals with 0.2 ml glacial acetic acid as a preservative. The urine samples were treated with kaolin and filtered; with authentic purified reference compounds as standards, the amount of dioxane and p-dioxane-2-one present in the urine was determined by analytical GC as described previously (31). Creatinine was determined by the modified micromethod of Folin's creatinine assay (13).

The separation of 14C-labeled metabolite was achieved by ion-exchange chromatography. The urine samples obtained from rats given [14C]dioxane (3 g/kg; 500 μCi/kg) were alkalinized to pH > 12 with 10% NaOH, a treatment that converts p-dioxane-2-one into 2-hydroxyethoxyacetate. The alkalinized urine (0.2 to 0.5 ml) was then applied to anion-exchange columns (Bio-Rad AG1x8, acetate form, 200 to 400 mesh, 0.7 x 4 cm) and eluted with 9.5 ml distilled water (nonanionic fraction) followed by 10 ml 0.5 M NaCl (anionic fraction). Aliquots (0.5 ml) of the fractions were mixed with Bray's solution (10 ml) for liquid scintillation counting. Over 98% of the radioactivity applied to the column could be recovered in these 2 fractions. The radioactivity was present mainly in the form of p-dioxane in the nonanionic fraction and almost exclusively as 2-hydroxyethoxyacetate in the anionic fraction. The radioactivity was measured in a liquid scintillation spectrometer (Beckman LS-233) with the external standardization method for quench correction. Using the initial specific activity of [14C]dioxane, the radioactivity present in the metabolite fraction was converted to μmol and expressed as mg p-dioxane-2-one.

Acute toxicity studies were carried out with rats weighing 130 to 170 g. Dioxane and p-dioxane-2-one were dissolved in 0.9% NaCl solution and administered i.p. After preliminary studies, a minimum of 7 doses was administered for the determination of each LD50. For each dose at least 10 rats were used, with 20 to 30 rats used for doses close to...
the LD₅₀'s. The doses ranged from 4.0 to 5.8 g/kg body weight for dioxane and 0.5 to 1.2 g/kg for p-dioxane-2-one. The LD₅₀'s with fiducial limits at 95% probability, based on deaths occurring 48 hr after the administration, were calculated from probit regression lines according to the method of Burn et al. (7).

RESULTS

The effect of inducers and inhibitors of MFO's on the in vivo metabolism of dioxane in the rat was studied with both nonradioactive and ¹⁴C-labeled dioxane. Three types of inducers (namely, PB, PCB, and MC) were used. Data in Chart 1, A and B, show the effect of PCB and MC on the excretion of dioxane and its principal urinary metabolite, p-dioxane-2-one, as measured by analytical GC. These data along with those showing PB effect (Ref. 32, Fig. 1) indicate that all 3 types of inducers increased the total amount of the metabolite excreted. In addition, the time of onset of peak excretion was reduced from between 20 to 28 hr for the control rats to about 12 hr for rats pretreated with inducers. Of the 3 types of inducers, PB had the greatest effect, followed by PCB; MC was the weakest. Administration of DPEA, a long-acting in vivo inhibitor of MFO's (21, 22), partially blocked the stimulatory effect of PCB (Chart 1A) and of PB (data not shown) during the first 16 to 24 hr following dioxane administration. DPEA alone was also inhibitory (Chart 1C). Pretreatment of rats with cobaltous chloride, which inhibits the synthesis of cytochrome P-450 (26), also decreased the excretion of the metabolite (Chart 1C). Both inhibitors delayed the time of peak excretion.

To ensure that the stimulatory and inhibitory effects observed above were not due to the modulation of renal excretory function by various agents, we determined the amount of creatinine excreted during each collecting period; as found previously (32), administration of dioxane caused decrease of creatinine excretion during the first 8 hr. When the results were calculated as mg p-dioxane-2-one per mg creatinine, they were found to be in good agreement with those shown in Chart 1. PB, PCB, and, to a lesser extent, MC all increased the metabolism of dioxane and its toxicity, we determined the acute toxicity of dioxane and its metabolite. The metabolite was considerably more toxic than was its parent compound; the LD₅₀ was 0.79 ± 0.15 g/kg body weight for p-dioxane-2-one and 5.3 ± 0.1 g/kg for dioxane. Pretreatment of rats with PCB increased the acute toxicity of dioxane (LD₅₀, 4.4 ± 0.1 g/kg). The MFO inhibitor, DPEA, was protective when administered to PCB-pretreated rats; at the dioxane dose of 4.55 g/kg, the mortality rate was decreased from 70% for PCB-pretreated rats to 30% for rats pretreated with PCB and given DPEA together with dioxane. Pretreatment of rats with MC or PB had no significant effect on the acute toxicity of dioxane; the LD₅₀ after pretreatment was 5.2 ± 0.1 g/kg for MC and 5.4 ± 0.2 g/kg for PB. The effect of pretreatment with inducers on the acute toxicity of p-dioxane-2-one was tested at a lactone dose of 0.7 g/kg. The results shown in Table 2 suggest that PCB and MC increase whereas PB decreases the toxicity of the lactone.

DISCUSSION

The results presented substantiate the involvement of MFO's in the in vivo metabolism of dioxane. Typical in-

![Table 1](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Metabolite excreted (mg p-dioxane-2-one/200 g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12 hr</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>57.2 ± 8.2p</td>
</tr>
<tr>
<td>PCB</td>
<td>168.3 ± 24.6c</td>
</tr>
<tr>
<td>MC</td>
<td>110.1 ± 0.1d</td>
</tr>
<tr>
<td>PB</td>
<td>180.6 ± 2.5e</td>
</tr>
<tr>
<td>12-24 hr</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>88.0 ± 14.8</td>
</tr>
<tr>
<td>PCB</td>
<td>106.0 ± 1.8</td>
</tr>
<tr>
<td>MC</td>
<td>102.6 ± 4.5</td>
</tr>
<tr>
<td>PB</td>
<td>134.9 ± 11.3</td>
</tr>
<tr>
<td>24-36 hr</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>111.7 ± 27.0</td>
</tr>
<tr>
<td>PCB</td>
<td>54.0 ± 3.0</td>
</tr>
<tr>
<td>MC</td>
<td>73.8 ± 5.5</td>
</tr>
<tr>
<td>PB</td>
<td>45.6 ± 1.4</td>
</tr>
<tr>
<td>36-48 hr</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32.5 ± 12.9</td>
</tr>
<tr>
<td>PCB</td>
<td>32.1 ± 9.2</td>
</tr>
<tr>
<td>MC</td>
<td>27.1 ± 8.0</td>
</tr>
<tr>
<td>PB</td>
<td>5.2 ± 3.7</td>
</tr>
</tbody>
</table>

a For comparison, the corresponding values, determined during the same time periods by analytical GC, were: 52.1, 80.4, 95.1, and 35.5.

b Mean ± S.E.

c p < 0.05.

d p < 0.01.

e p < 0.001.

![Image](https://example.com/image.png)
The relationship between the in vivo metabolism of dioxane and its toxicity has been explored in view of the possibility that p-dioxane-2-one may be carcinogenic. A number of saturated and unsaturated lactones are known to be carcinogenic (10, 28). Acute toxicity studies revealed that p-dioxane-2-one is substantially more toxic than is dioxane. Agents that modify the metabolism of dioxane may therefore be expected to modify its toxicity; this is in fact the case for certain inducers and inhibitors. Pretreatment with PCP, which increased the metabolism of dioxane, also increased its toxicity. Concomitant treatment with DPEA, which curtailed the stimulatory effect of PCP on metabolism, protected PCP-pretreated rats against increased dioxane toxicity. Pretreatment with MC, which had only small effect on the metabolism, had no significant effect on the LD₅₀ of dioxane. PB, however, appears to be an exceptional case, since PB pretreatment had no effect on the LD₅₀ of dioxane in spite of substantially increasing its metabolism to p-dioxane-2-one.

The apparent lack of PB effect on the toxicity of dioxane suggests that p-dioxane-2-one requires further metabolism to exert its toxic effect. This possibility has been tested. Both PB and MC increased the toxicity of p-dioxane-2-one, whereas PB appeared to have the opposite effect. Thus, the totality of the results suggests that the generation of toxic substance from dioxane may involve a multistep mechanism. The first stage would involve the biotransformation of dioxane to p-dioxane-2-one by 1 of the possible mechanisms described previously (30, 31); PB, PCP, and, to a much lesser extent, MC stimulate this stage. P-Dioxane-2-one is then further metabolized (possibly by desaturation of the ring) to yield a toxic intermediate. This further metabolism may be increased by PCP or MC pretreatment but not by PB. Additional studies on the metabolism of p-dioxane-2-one and the identification of the toxic intermediate are needed before this hypothesis can be established.

The relationship between the metabolism and toxicity of dioxane and the influence of enzyme inducers on this relationship are of great interest in view of the wide use of this solvent. Indeed, metabolic processes underlying acute toxicity and carcinogenicity have often been found to be closely related (14, 17, 23, 25, 29). Individuals exposed simultaneously to dioxane vapor and enzyme inducers in the environment and/or in the diet may be expected to respond differently depending on the type of inducer. Hence, further elucidation of the mechanism of the toxicity of dioxane may eventually provide information on the environmental and dietary factors that should be avoided by individuals exposed to this solvent.

### ACKNOWLEDGMENTS

The authors are indebted to Sherry Klumpp, Sandra R. Martinez, Melanie Amadon, Gary M. Williamson, Michael Newchurch, and especially Georgia M. Bryant for invaluable assistance in carrying out the acute toxicity studies. We thank the Monsanto Chemical Co., St. Louis, Mo., for the gift of Aroclor 1254 through the courtesy of William B. Papageorge.

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