Reduced Nicotinamide Adenine Dinucleotide Phosphate-dependent Formation of 2,3-Dihydro-2,3-dihydroxyaflatoxin B₁ from Aflatoxin B₁ by Hepatic Microsomes¹

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ABSTRACT

2,3-Dihydro-2,3-dihydroxyaflatoxin B₁ (dihydrodiol) was formed as a major metabolite in the incubation of aflatoxin B₁ with rat and hamster liver microsomes. The yield of the dihydrodiol was maximal at pH 6.5, was reduced nicotinamide adenine dinucleotide phosphate- and cytochrome P-450-dependent, and was increased 2- to 4-fold by pretreatment of the animals with phenobarbital; pretreatment with 3-methylcholanthrene did not alter the activity of rat hepatic microsomes. Inhibitors of epoxide hydrase did not lower the yield of the dihydrodiol in these systems. Negligible yields of the dihydrodiol were formed from aflatoxin B₂, and rat liver microsomes in the presence of DNA. Little or no formation of the dihydrodiol was noted with microsomes from rat intestinal mucosa, kidney, or lung.

These results further support the formation of aflatoxin B₁, 2,3-oxide as a major electrophilic metabolite of aflatoxin B₁ in rat and hamster liver microsomal systems, since this highly reactive epoxide would be expected to hydrolyze readily to form the dihydrodiol.

INTRODUCTION

Considerable evidence indicates that AFB₁, 2,3-oxide⁴ is an ultimate carcinogenic, mutagenic, and electrophilic metabolite of the potent hepatocarcinogenic mycotoxin AFB₁ (Refs. 2 and 16, and references therein). The metabolic formation of this reactive epoxide is supported by the release of 2,3-dihydro-2,3-dihydroxy-AFB₁ (also referred to herein as the dihydrodiol) (Chart 1) (23–25) and 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B₁ (3, 4, 16, 17) as major products of the weak acid hydrolysis of nucleic acid-AFB₁ adducts formed in the rat liver in vivo or by hamster and rat liver microsomes in vitro. However, since it has not been possible to isolate AFB₁, 2,3-oxide from either in vitro metabolic systems or from chemical syntheses (Refs. 5, 7, and 10; D. H. Swenson, J. A. Miller, and E. C. Miller, unpublished data), this epoxide appears to have a very short half-life in aqueous systems and, in the absence of strong nucleophiles, would be expected to hydrolyze to 2,3-dihydro-2,3-dihydroxy-AFB₁. Thus, microsomal epoxidation of AFB₁ in the absence of added nucleic acids should yield the dihydrodiol, and the amounts of the latter product should reflect the epoxidative activity of the system (Chart 1).

Patterson and Roberts (21) and Gurtoo and Campbell (13) have reported the formation of a microsome- and NADPH-dependent metabolite of AFB₁, which they designated as AFB₁Δ₂, the hydration product of AFB₁ under acidic conditions (Chart 1). A requirement for NADPH for hydration of the 2,3-double bond of AFB₁ is not consistent with other known enzymatic hydrations; in fact, Schabort and Steyn (22) reported that NADPH was not necessary for this reaction. The characterization of AFB₁Δ₂ as the reaction product by the former authors was based on the UV spectrum of the product and on its reactions with proteins. However, the UV spectra of aflatoxin B₁ and the dihydrodiol are indistinguishable (25), and they react with proteins in the same fashion to give similar products (1, 26). In view of this situation, we have reinvestigated the identity of the product formed on incubation of AFB₁ with NADPH-fortified hepatic microsomes in the absence of strong nucleophiles and have characterized it as 2,3-dihydro-2,3-dihydroxy-AFB₁. The formation of this dihydrodiol under various conditions has been examined.

MATERIALS AND METHODS

Instrumentation and General Procedures. UV spectra were determined on a Beckman DB spectrophotometer equipped with a Sargent recorder. The Varian CH-7 mass spectrometer was equipped with a digital mass marker (Varian Associates, Palo Alto, Calif.) and a direct insertion probe (Variset Corp., Madison, Wis.); it was standardized with perfluorokerosene (Penninsullar Chem Research, Inc., Gainesville, Fla.).

High-performance liquid chromatography (hereafter referred to as liquid chromatography) was accomplished with a μBondapak C₄ reverse-phase column on a Waters ALC 201 liquid chromatograph equipped with a Model U6K injector system, a Model 600 solvent programmer (all products of Waters Associates, Milford, Mass.), and an SF 770 variable wavelength photometer set at 360 nm (Schoeffel Instrument Co., Westwood, N. J.). Unless otherwise noted, all liquid chromatography was carried out with an isocratic system of ethanol:water:glacial acetic acid (5:15:0.001, by volume) with a flow rate of 1 ml/min.

Disposable vinyl gloves were used in handling the aflatox-
Chart 1. The proposed route of formation of the dihydrodiol on incubation of NADPH-fortified liver microsomes with AFB. The steri configuration of the 2,3-oxide follows from recent studies on the structures of the principal adducts formed on incubation of AFB with nucleic acids by liver microsomes (4, 16). AFB\(_2\) is probably racemic at C-2 because of the ease of opening and closing of the terminal tetrahydrofuran ring in neutral aqueous media (1, 21, 26). In the dihydrodiol this ring behaves similarly, but steric hindrance by the hydroxyl group at C-3 may favor the formation of the trans-diol (shown in the chart) as the major isomer. On the other hand, formation of the acetonide of the dihydrodiol (see "Results") implies a cis-diol structure which presumably results from opening and closing of the terminal ring in the dihydrodiol.

 ins, and aflatoxin residues were decontaminated with 5% sodium hypochlorite.

**Chemicals.** AFB was purchased from Calbiochem (La Jolla, Calif.). 2,3-Dihydro-2,3-dihydroxy-AFB\(_2\) was synthesized as described previously (25, 26) and purified by thin-layer chromatography on silica and/or by liquid chromatography. 2-[(2,4-Dichloro-6-phenyl)phenoxy]ethylamine and SKF-525A were generously provided by Dr. R. E. McMahon (Lilly Research Laboratories, Indianapolis, Ind.) and by Smith, Kline, and French Laboratories (Philadelphia, Pa.), respectively. DNA (calf thymus, type I), glucose 6-phosphate, NADP\(^+\), and NAD\(^+\) were purchased from Sigma Chemical Co. (St. Louis, Mo.); glucose 6-phosphate dehydrogenase was obtained from Worthington Biochemicals Corp. (Freehold, N. J.). Styrene oxide and 3,3,3-trichloro-propylene oxide were purchased from the Aldrich Chemical Co. (Milwaukee, Wis.).

**Animals.** Adult male Fischer rats (175 to 200 g) were obtained from the Charles River Breeding Co., Wilmington, Mass.; male Syrian golden hamsters (about 100 g) were purchased from ARS/Sprague-Dawley (Madison, Wis.). The animals were fed Wayne Breeder Blox (Allied Mills Inc., Chicago, Ill.). Where specified, they were given drinking water that contained 0.1% sodium phenobarbital (J. T. Baker Chemical Co., Phillipsburg, N. J.) for 1 week prior to being killed. Some rats were given i.p. injections of 20 mg of 3-methylcholanthrene (Eastman Kodak Co., Rochester, N. Y.) per kg body weight per 2 ml of sterile tricoctaino (Sigma) 48 and 24 hr before sacrifice.

The animals were killed by decapitation, and the tissues were immediately collected and placed on ice. The lumen of the small intestine was rinsed with 0.9% NaCl solution, and the mucosa was obtained by scraping with a spatula. The tissues were homogenized with a Potter-Elvehjem apparatus in 3 volumes of 0.25 m sucrose solution containing 5 mm Tris-HCl buffer, pH 8.0. The supernatant from centrifugation of the homogenate at 13,000 \(\times\) g for 10 min was centrifuged at 43,000 \(\times\) g for 60 min. The small contaminating mitochondrial pellet underlying the microsomal pellet was discarded, and the microsomes were resuspended in the homogenization medium (1 g of liver per ml). In some cases the suspensions of liver microsomes were lyophilized and the vials were sealed under vacuum; under these conditions the hepatic microsomes retained 80% or more of their activity for at least 2 months at -20°.

**Assay Conditions.** Unless otherwise specified, the reaction mixture (2 ml) contained the following ingredients: 0.075 m potassium phosphate buffer, pH 6.5; 0.003 m MgCl\(_2\); 0.00025 m NAD\(^+\); 0.00025 m NADP\(^+\); 0.0063 m glucose 6-phosphate; 0.002 m EDTA; 2.5 units of glucose-6-phosphate dehydrogenase; 200 \(\mu\)g of AFB\(_2\) in 0.02 ml of dimethyl sulfoxide; and microsomes from 60 mg of liver. The reaction mixture, except for the last 2 ingredients, was preincubated for 3 min, the AFB\(_2\) was added, and then the reaction was started by the addition of the microsome suspension. The reaction mixture was shaken in air at 37°, and the usual reaction time was 10 min. The reactions were stopped by placing the flasks in an ice bath and adding 0.15 ml of 2 m NaCl and 4 ml of cold ethanol. After 30 min the mixtures were centrifuged. The supernatant was filtered through a 0.45-\(\mu\)m Millipore filter (Millipore Corp., Bedford, Mass.) and used directly for liquid chromatography. The liquid chromatograph was calibrated with synthetic dihydrodiol [assumed molar absorbance at 360 nm of 2.2 \(\times\) 10\(^4\) (26), and the amounts of metabolically formed dihydrodiol were determined from the peak heights at the retention time of the dihydrodiol. The metabolite for formation of the acetone (25) and for UV spectroscopy was obtained by repetitive liquid chromatography of aliquots from the incubation mixture.

In some cases 4 mg of DNA were added to the above assay mixture. In these cases the volume of the reaction mixture was 3 ml, and the microsomes were sedimented by centrifugation before addition of the ethanol and NaCl. The DNA was collected by centrifugation and redissolved in 3 ml of 0.015 m NaCl-0.0015 m sodium citrate, pH 7.0, at 4°. The DNA was hydrolyzed at 100° in 0.15 m HCl for 30 min, and the hydrolysis products were determined by liquid chromatography (16).

**RESULTS**

Preliminary Studies and Identification of a Major Microsomal Metabolite as 2,3-Dihydro-2,3-dihydroxy-AFB.

Preliminary studies in which AFB was incubated at pH 6.5 with microsomes from phenobarbital-treated rat or hamster livers showed the presence of a major metabolite that chromatographed at the retention time of 2,3-dihydro-2,3-dihydroxy-AFB\(_2\) and little or no product with the retention time of aflatoxin B\(_2\).

Evidence for the identity of the metabolite as 2,3-dihydro-2,3-dihydroxy-AFB\(_2\) was obtained by its cochromatography with the synthetic compound on a \(\mu\)Bondapak column with 4 solvent systems (Table 1). The product was further identified as the dihydrodiol by the identities of its absorption spectra in methanol and in alkaline methanol with those of the synthetic dihydrodiol (Chart 2). Finally, the metabolite formed an acetone that had the same mole peak (m/e =
Retention times of synthetic 2,3-dihydro-2,3-dihydroxy-AF\(\text{B}_1\) and the metabolite of AF\(\text{B}_1\), formed by normal hamster liver microsomes

All chromatography was carried out on a µBondapak C\(_8\) reverse-phase column. All of the solvents were prepared by volume measurements.

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Retention time (min)</th>
<th>Synthetic compound</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% ethanol:water:glacial acetic acid (5:15:0.001)</td>
<td>1</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>0.01% acetic acid in methanol:water (3:7)</td>
<td>1</td>
<td>18.0</td>
<td>17.8</td>
</tr>
<tr>
<td>Ethanol:1-propanol:water (10:5:85)</td>
<td>1</td>
<td>23.6</td>
<td>23.6</td>
</tr>
<tr>
<td>25% Methanol:88% formic acid (100:0.02)</td>
<td>2</td>
<td>18.9</td>
<td>18.9</td>
</tr>
</tbody>
</table>

![Chart 2](chart.png)

Chart 2. The UV absorption spectra of synthetic 2,3-dihydro-2,3-dihydroxy-AF\(\text{B}_1\), and of the metabolite formed from AF\(\text{B}_1\), by hamster liver microsomes in the absence of added nucleophile. Top spectra were taken in methanol; bottom spectra were taken after the addition of 0.1 ml of 1 N NaOH per 0.9 ml of methanol solution.

386) and fragments (\(m/e = 371, 357, 328, 311, 299, 283, \) and 271) as shown by the synthetic compound (25).

**Reaction Conditions for Formation of 2,3-Dihydro-2,3-dihydroxy-AF\(\text{B}_1\)**. The maximum yield of dihydrodiol was obtained when the incubations were carried out at about pH 6.5; the yields obtained at pH 6.1, 6.2, 6.3, and 7.1, respectively, were approximately 35, 60, 75, and 30% of those obtained at pH 6.5. This finding was consistent with the very poor recoveries of the dihydrodiol when it was added to the incubation mixture in place of AF\(\text{B}_1\) at pH’s of 7.0 or greater. With 20-min incubations and hepatic microsomes from phenobarbital-treated hamsters, the recoveries of 20 nmol of the dihydrodiol were 100, 88, 11, and 8% for incubations carried out at pH 6.0, 6.7, 7.1, and 7.5, respectively.

The reaction was dependent on the addition of NADP\(^+\), glucose 6-phosphate plus glucose-6-phosphate dehydrogenase, and undenatured microsomes (Table 2). The reaction was linear for at least 10 min when microsomes from 60 mg of liver from normal or phenobarbital-treated rats or hamsters were added per 2-ml reaction mixture. For 10-min incubations linearity with respect to enzyme concentration was observed with microsomes from 10 to 60 mg of liver per flask; the system was saturated by 200 µg of AF\(\text{B}_1\) per flask.

The reaction was inhibited by cytochrome P-450 oxidase inhibitors (Table 3). One \(\times 10^{-3}\) M SKF-525A caused a 60 to 70% decrease in activity, and 2-[(2,4-dichloro-6-phenyl)phenoxy]ethylamine (15, 20, 28, 29) gave a similar level of inhibition when added at a level of \(1 \times 10^{-4}\) M. Neither 3,3,3-trichloropropylene oxide nor styrene oxide, both inhibitors of epoxide hydrase (19), caused appreciable inhibition.

Addition of 4 mg of DNA per flask reduced the yields of the dihydrodiol to negligible levels (<0.5 nmol) for incubations containing hepatic microsomes from normal or phenobarbital-treated rats or hamsters. Hydrolysis of the DNA from the incubation mixtures yielded 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B\(_1\) (4, 16, 17); little or no dihydrodiol was detected in these hydrolysates. The yields of the guanyl derivative, when corrected for the 50% recovery of the DNA from the incubation mixture, were 70 to 80% of the yields of the dihydrodiol that were obtained in the absence of the DNA.

**Yields of 2,3-Dihydro-2,3-dihydroxy-AF\(\text{B}_1\), with Microsomes from Various Tissues.** Microsomes from 60 mg of normal adult male rat liver converted about 5 nmol of the 640 nmol (200 µg) of AF\(\text{B}_1\) added to the incubation mixture to the dihydrodiol during a 10-min incubation. The yields of

<table>
<thead>
<tr>
<th>Modification</th>
<th>Normal liver</th>
<th>Phenobarbital-treated liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>-NADP(^+)</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>-NAD(^-)</td>
<td>105</td>
<td>110</td>
</tr>
<tr>
<td>-Glucose 6-phosphate and glucose-6-phosphate dehydrogenase</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>-Mg(\text{Cl}_2)</td>
<td>95</td>
<td>110</td>
</tr>
<tr>
<td>Microsomes heated at 90(^\circ), 10 min</td>
<td>≤5</td>
<td>5</td>
</tr>
</tbody>
</table>

Yields of 2,3-Dihydro-2,3-dihydroxy-AF\(\text{B}_1\), on incubation of AF\(\text{B}_1\), with rat liver microsomes under various conditions

Liver microsomes from male Fischer rats that were either untreated or given 0.1% sodium phenobarbital in the drinking water for 1 week prior to assay were incubated in the standard system (see "Materials and Methods") except for the omissions indicated. The yields of dihydrodiol for the normal and phenobarbital-treated rat liver microsomes with the complete incubation mixture were 4.0 and 18.8 nmol, respectively.

**Table 2**

Yields of 2,3-dihydroxy-AF\(\text{B}_1\), on incubation of AF\(\text{B}_1\), with rat liver microsomes under various conditions

Liver microsomes from male Fischer rats that were either untreated or given 0.1% sodium phenobarbital in the drinking water for 1 week prior to assay were incubated in the standard system (see "Materials and Methods") except for the omissions indicated. The yields of dihydrodiol for the normal and phenobarbital-treated rat liver microsomes with the complete incubation mixture were 4.0 and 18.8 nmol, respectively.

% of 2,3-dihydro-2,3-dihydroxy-AF\(\text{B}_1\), obtained with complete incubation medium

<table>
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<th>Modification</th>
<th>Normal liver</th>
<th>Phenobarbital-treated liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>-NADP(^+)</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>-NAD(^-)</td>
<td>105</td>
<td>110</td>
</tr>
<tr>
<td>-Glucose 6-phosphate and glucose-6-phosphate dehydrogenase</td>
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<td>5</td>
</tr>
<tr>
<td>-Mg(\text{Cl}_2)</td>
<td>95</td>
<td>110</td>
</tr>
<tr>
<td>Microsomes heated at 90(^\circ), 10 min</td>
<td>≤5</td>
<td>5</td>
</tr>
</tbody>
</table>
The yields of dihydrodiol for the normal and phenobarbital-treated rat liver microsomes were about 5 times as great with hamster as with rat liver microsomes (Table 4). Phenobarbital pretreatment (0.1% in the drinking water for 1 week) of the animals increased the activities of the hepatic microsomes from each of these species at least 2-fold. Hepatic microsomes from rats pretreated with 3-methylcholanthrene had activities similar to those from untreated rats. Microsome preparations from the kidneys, small intestinal epithelium, and lungs of normal adult male rats yielded less than 0.5 nmol of the dihydrodiol per 60 mg of tissue with a 10-min incubation.

**DISCUSSION**

Earlier studies have provided strong presumptive evidence for the metabolic formation of AFB₁, 2,3-oxide by rat and hamster liver microsomes. Thus, Swenson et al. (24, 25) characterized the dihydrodiol as a major product formed on weak acid hydrolysis of DNA- and rRNA-aflatoxin B₁ adducts formed either in vitro or in vivo and isolated by a phenol procedure. More recent studies from our laboratory (16) and those of Croy et al. (3) and Essigmann et al. (4) and of Martin and Garner (17) have characterized the major product formed on acid degradation of the nucleic acid adducts formed either in vitro or in vivo and protected from any contact with weak alkali as 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B₁, this is the expected product for attack by aflatoxin B₁, 2,3-oxide on the 7-nitrogen of guanine residues. Our isolation of 2,3-dihydro-2,3-dihydroxy-AFB₁, the solvolysis product of the epoxide, from incubations of NADPH-fortified rat or hamster liver microsomes in the absence of an added nucleophile now provides more direct evidence for the metabolic formation of the 2,3-oxide. The yields of the dihydrodiol were maximal when the incubations were carried out at pH 6.5; added dihydrodiol was readily destroyed in incubations at pH 7.0 or greater. Garner (5) earlier failed to find the dihydrodiol after incubation of AFB₁ and hamster liver microsomes at pH 7.4 but did find 2 water-soluble metabolites identical with 2 degradation products of the dihydrodiol under these conditions.

Addition of 3,3,3-trichloropropylene oxide or styrene oxide, both of which are epoxide hydrase inhibitors (19), had little effect on the formation of the dihydrodiol by rat liver microsomes. Likewise, Gurtoo and Bejba (12) have noted that the microsome-induced binding of AFB₁ to DNA is not influenced by the epoxide hydrase inhibitor cyclohexene oxide. Garner and Wright (8) showed that the same inhibitor did not influence the lethality of AFB₁ for Salmonella typhimurium TA1530 in the presence of liver microsomes. These results and our present data suggest that the hydrolysis of the epoxide is not dependent on the microsomal epoxide hydrase, either because the epoxide is so easily hydrolyzed that the enzyme cannot appreciably facilitate the reaction or because the epoxide is not a good substrate for the enzyme. For example, stereoisomers of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, which are strongly electrophilic metabolites of benzo(a)pyrene, are poor substrates for epoxide hydrase (27). Similarly, the microsomal-mediated binding of the model furan compound 2-(N-ethylcarbamoylhydroxymethyl)furan to nucleophiles through a presumed intermediate epoxide was not inhibited by styrene oxide, cyclohexene oxide, or 3,3,3-trichloropropylene oxide (11).

In view of the data presented herein, we conclude that the NADPH-dependent metabolite of AFB₁, studied by Patterson and Roberts (21) and by Gurtoo and Campbell (13) was 2,3-dihydro-2,3-dihydroxy-AFB₁, rather than AFB₁a (2,3-dihydro-2-hydroxy-AFB₁). The latter 2 compounds are not distinguishable by the criteria studied by these authors. In spite of the high capacities of the hepatic microsomes from hamsters and phenobarbital-treated rats to form AFB₁, 2,3-oxide, as measured by the yields of the dihydrodiol or of aflatoxin-nucleic acid adducts (Refs. 5, 16, and 25; this paper), these animals are less susceptible to hepatic carcinogenesis by AFB₁ than are non-phenobarbital-treated rats (14, 18, 23). Hamsters and phenobarbital-treated rats also form less hepatic nucleic acid-bound 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B₁ adducts in vivo than do control rats on administration of AFB₁ (Refs. 6, 9, and 23; M. M. 2,3-Dihydro-2,3-dihydroxyaflatoxin B₁

<table>
<thead>
<tr>
<th>Species</th>
<th>Pretreatment</th>
<th>Dihydrodiol* (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>None</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Rat</td>
<td>Phenobarbital</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Rat</td>
<td>3-Methylcholanthrene</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Hamster</td>
<td>None</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Hamster</td>
<td>Phenobarbital</td>
<td>65 ± 6</td>
</tr>
</tbody>
</table>

* nmol of dihydrodiol formed per 60 mg of liver per 10 min incubation. Values are averages ± S.D. for analyses on 3 livers.

(Ref. 5) earlier failed to find the dihydrodiol after incubation of AFB₁ and hamster liver microsomes at pH 7.4 but did find 2 water-soluble metabolites identical with 2 degradation products of the dihydrodiol under these conditions.

Table 3

**Effects of inhibitors of cytochrome P-450 oxidase and of epoxide hydrase on the yields of 2,3-dihydro-2,3-dihydroxy-AFB₁**

The assays were carried out as described for the standard assay (see "Materials and Methods"); the incubation time was 10 min.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Molarity</th>
<th>Normal rat</th>
<th>Pretreated rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKF-525A</td>
<td>1 x 10⁻⁴</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2-[(2,4-Dichloro-6-phenyl)phenoxo]ethylamine</td>
<td>1 x 10⁻⁴</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>3,3,3-Trichloropropylene oxide</td>
<td>1 x 10⁻³</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Styrene oxide</td>
<td>1 x 10⁻³</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4

**Yields of 2,3-dihydro-2,3-dihydroxy-AFB₁ on incubation of AFB₁ with rat and hamster liver microsomes**

Incubations were carried out as described in "Materials and Methods."
Moore, J. M. Shade, E. C. Miller, and J. A. Miller, unpublished data). Assuming, as seems very likely, that AFB, 2,3-oxide is a major ultimate carcinogenic metabolite, the capacity of the microsomal enzymes to form this epoxide would be only one of the factors that determine the hepatocarcinogenicity of AFB,. Alternative routes of metabolism of AFB, (e.g., demethylation, hydroxylation at various sites) (2) must limit the amounts of the epoxide formed under nonsaturating levels of AFB, in vivo.

REFERENCES

20. Parli, C. J., Lee, N. W., and McMahon, R. E. The Relationship between the Metabolism, of 2,4-Dichloro-6-phenylisoxazolyl (DPEA) and Related Compounds and Their Activities as Microsomal Mono-oxygenase Inhibitors. Drug Metab. Disposition, 1: 628-633, 1973.
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