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Aflatoxin B1 metabolism in the rat: polyhalogenated biphenyl enhanced conversion to aflatoxin M1

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1. The effects of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) on the formation *in vitro* of aflatoxin Q1 and aflatoxin M1 from aflatoxin B1 by rat-liver microsomes were investigated.

2. AFB1 metabolism by hepatic microsomes from PBB- and PCB-treated rats resulted in 16- and 30-fold increases, respectively, in levels of aflatoxin M1. The enhanced formation of aflatoxin M1 did not correlate with PBB and PCB stimulation of benzo[*a*]pyrene hydroxylase (AHH) activity.

3. Studies *in vivo* clearly demonstrated enhanced secretion of aflatoxin M1 by female lactating rats with prior exposure to PCBs. PCB pretreatment enhanced the activity of mammary as well as hepatic tissue microsomal preparations in converting aflatoxin B1 to aflatoxin M1.

4. Our findings indicate that PCB exposure increases the production of aflatoxin M1 *in vitro* and also increases the levels of aflatoxin M1 released into the milk.

Introduction

Aflatoxin B1 is a potent hepatotoxin and hepatocarcinogen produced as a secondary metabolite by *Aspergillus* molds (Wogan and Newberne 1967). Authentic aflatoxin B1 is metabolized in mammalian systems to a variety of metabolites with the relative levels of each dependent on factors such as sex (Righter *et al.* 1972, Gurtoo and Motycka 1976), species (Roebuck and Wogan 1977), hormonal status (Patterson and Roberts 1972, Chedid, Halfman and Greenberg 1980), diet (Mainigi and Campbell 1980) and chemical modulation (Gurtoo and Dahms 1979). Alteration of B1 metabolism may be evidenced as either a change in the rate of aflatoxin B1 metabolism, a shift in the preferred metabolic pathway (i.e. activation or detoxification), or a combination of both.

Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) are persistent environmental contaminants which have been identified as residues in human fat and breast milk. Both PCBs and PBBs are potent inducers of the cytochrome P-450-dependent mixed-function oxidase (MFO) system which also plays the major role in the metabolism of aflatoxin B1. Induction of hepatic MFO enzymes by polyhalogenated biphenyls may influence the rate of formation of aflatoxin metabolites and thus modulate their toxicity. Although the MFO enzymes responsible for the metabolism of aflatoxins are primarily found in the liver, studies have also shown that extrahepatic tissues such as kidney, lung and intestine are capable of MFO activity (Litterst *et al.* 1975).

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Aflatoxins are excreted in the milk of lactating animals; however, the role of mammary MFO enzymes in determining the spectrum of aflatoxin B1 and its metabolites in these milk samples is not known. It has been shown that pretreatment of rats with PBBs and PCBs induces microsomal MFO activity in liver and extrahepatic tissues, including mammary tissue (Dent *et al.* 1977 a, McCormack *et al.* 1979). Induced mammary MFO activity, measured as benzo[*a*]pyrene hydroxylase, was significantly increased over control values; however, enzyme activities were less than 1% of that observed in the liver.

This study reports the effects of pretreatment of male and lactating female rats with commercial mixtures of PCBs (Aroclor 1254) and PBBs (FireMaster BP-6) on the metabolism *in vitro* of aflatoxin B1 by microsomes isolated from liver, kidney and mammary tissue. In addition the effect of PCBs on the secretion *in vivo* of aflatoxins in the milk of lactating females given a sub-lethal dose of aflatoxin B1 was studied.

Materials and methods

Chemicals

Aflatoxins B1, M1 and Q1 were obtained from Sigma Chemical Co. (St Louis, MO, USA). Aflatoxin B1 (uniformly labelled with tritium; S.A. 15 Ci/mmol; radiochemical purity, 98%) was purchased from Moravek Chemicals (City of Industry, CA, USA). Sodium phenobarbital (Harvey Laboratories, Inc., Philadelphia, PA, USA) was diluted in saline to a conc. of 80 mg/ml. 3-Methylcholanthrene (Sigma Chemical Co., St Louis, MO, USA) was dissolved in corn oil to give a conc. of 30 mg/ml. Polychlorinated biphenyls, Aroclor 1254 (Monsanto Chemical Co., St Louis, MO, USA) were diluted in corn oil to give a conc. of 176 mg/ml. Polybrominated biphenyls, FireMaster BP-6 (Velsicol Chemical Co., St Louis, MI, USA) were diluted in corn oil to give a conc. of 100 mg/ml. NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, $MgCl_2$ and benzo[*a*]pyrene were obtained from Sigma Chemical Co. (St Louis, MO, USA). 4-Dimethylaminoantipyrine was supplied by Aldrich Chemical Co. (Milwaukee, WI, USA). [H^3]Benzo[*a*]pyrene was obtained from Amersham Corp. (Arlington Heights, IL, USA) (sp. activity 70 Ci/mmol) and purified to 96·5% via Florisil column chromatography. Female rats were anaesthetized using halothane gas (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA). Milk secretion was stimulated with synthetic oxytocin (VEDCO, Inc., Omaha, NE, USA).

Animals

Adult male Sprague–Dawley rats (body wt. 300–320 g) were obtained from Timco, Inc. (Houston, TX, USA). All animals were housed in individual cages with feed and water *ad libitum*. No aflatoxin was detected in samples of rat chow (Ralston Purina Co., St. Louis, MO, USA) analysed before initiation of experiments. All test animals were acclimatized to their new surroundings and diet for four days before administration of the test chemicals and corn oil. Pregnant Sprague–Dawley rats (body wt. 400–450 g), day 16 of gestation, were obtained from Timco, Inc. (Houston, TX, USA). One week after delivery the mothers were weight-matched, ear-tagged and the mothers plus litters randomly assigned to either a control or PCB treatment group.

Treatment with microsomal inducers

Male rats were weight-matched then randomly divided into five treatment groups, which were dosed over a three-day period according to the following schedule: group 1 served as negative controls and received no pretreatment; group 2 received a daily i.p. injection of phenobarbital (80 mg/kg) for three days; group 3 received 3-methylcholanthrene (30 mg/kg) daily for three days; group 4 received an i.p. injection of PBB (575 mg/kg) on day 1 of the experiment; group 5 was pretreated with PCBs (575 mg/kg) on day 1 of the experiment by the same method as group 4. Microsomal preparations for incubations *in vitro* were isolated on day 4 of the experiment.

To determine the effect of PCB pretreatment on milk flow, post-partum rats (one week) were divided into two groups—control and PCB treated. Control rats were treated with 1 ml of corn oil while the PCB group was treated with PCBs in corn oil at a dose of 575 mg/kg. Milk samples were collected on days 2 and 4 of the experiment. The volume of milk samples collected on days 2 and 4 were compared between control and PCB-treated rats. Microsomal preparations from control and PCB-pretreated lactating rats were isolated on day 4 for use in incubations *in vitro*.

The effect of PCB pretreatment on aflatoxin M1 levels in milk was determined by collecting a single milk sample at one hour post dosing with radiolabelled aflatoxin B1.

Enzyme assays in vitro

Microsomes were isolated from hepatic and renal tissue by the method of Lu and Levin (1972), thoroughly mixed, divided into 1 ml aliquots, and stored at −80°C until used. Protein content was determined by the method of Lowry *et al.* (1951).

Metabolism of aflatoxin B1 *in vitro* was carried out under conditions similar to those used by Roebuck, Siegel and Wogan (1978), using microsomes equivalent to 25 mg of protein.

Benzo[*a*]pyrene hydroxylase (BP) activity was determined radiometrically by quantifying the base-soluble metabolites following hexane extraction of the unchanged BP (DePierre *et al.* 1975, Nesnow, Fahl and Jefcoate 1977). The rate of oxidative *N*-demethylation of 4-dimethylaminoantipyrine was determined by quantifying the production of formaldehyde (Dewaide and Henderson 1968).

Metabolism of aflatoxin B1 in vivo *and excretion in milk*

Milk samples were collected using an apparatus in our laboratory (figure 1). Milk was suctioned into Teflon tubing (int. diam. 0·406 mm) leading to a collection vial held inside a sealed bottle. The Teflon tubing was changed following each milking to eliminate cross-contamination between radiolabelled milk samples from different animals.

Rats were milked one week after littering to allow milk production to reach maximum levels. Six hours before milking the mothers were isolated from the pups and anaesthetized in a chamber with halothane/air (1:19, v/v). The dams were then transferred to a modified anaesthesia cone that completely encased the head. The eyes were swabbed with a water-soluble lubricating jelly (K-Y Jelly, Johnson & Johnson, New Brunswick, NJ, USA) to prevent drying and irritation. Synthetic oxytocin (2 units) was injected i.p. and approx. three–five min later milk collection was initiated via suction of expelled droplets. Milk was collected for 15–20 min, yielding volumes of 0·5–1·0 ml.

Radioactivity of milk samples was measured in a scintillation counter after protein digestion. A 100 μl sample of milk was added to a 20 ml scintillation vial, 1 ml of Beckman Tissue Solubilizer-450 (Beckman Instruments, Inc., Fullerton, CA, USA) added and the sample allowed to digest overnight. Following digestion the pH was neutralized with 6 drops of glacial acetic acid, 10 ml of scintillation fluid (Beckman HP) added and the samples counted.

Aflatoxin analysis

Aflatoxins were extracted with chloroform and analysed by h.p.l.c. (Shepherd *et al.* 1982). Further confirmation of aflatoxin peaks was obtained by collection of the h.p.l.c. effluent via fraction collection and measurement of radioactivity by scintillation counting.

Statistics

Results were analysed statistically by analysis of variance. Each group was compared with its concurrent control for significance using Duncan's new multiple range test.

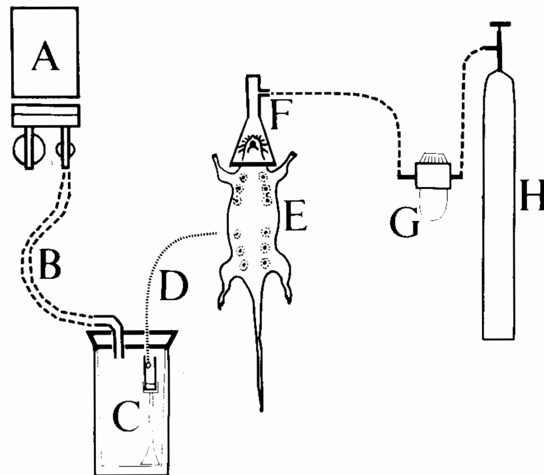

Figure 1. Schematic diagram of milk-collection apparatus.

The milk-collection apparatus consisted of: A, vacuum pump; B, vacuum line; C, vacuum bottle containing a glass collection vial; D, 0·406 mm Teflon collection line; E, anaesthetized rat; F, anaesthesia cone; G, anaesthesia regulator; H, oxygen supply.

Results

Effect of microsomal inducers on the metabolism of aflatoxin B1 in vitro

Table 1 summarizes the effects of pretreatment of male rats with PBBs and PCBs on the hepatic microsomal metabolism of aflatoxin B1 *in vitro*. Also shown in table 1 are the corresponding enzymic activities of two marker enzymes, aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase.

The control group (corn oil treatment) metabolized an average of $2{\cdot}5 \pm 1{\cdot}1$ nmol of AF-B1/25 mg microsomal protein in 30 min and produced two detectable metabolites, AF-Q1 (9-hydroxy aflatoxin B1) and AF-M1 (4-hydroxy aflatoxin B1) at levels of 0·16 and 0·5 nmol/25 mg microsomal protein in 30 min, respectively. Phenobarbital (PB) pretreatment resulted in an average 2·5-fold increase in aflatoxin B1 metabolized over that observed in the control rats; whereas, treatment with 3-MC produced a 4·5-fold increase in metabolism of AF-B1. Pretreatment with PBBs and PCBs resulted in an 8- and 17-fold increase, respectively, in AF-B1 metabolism by the induced microsomes compared with controls. Microsomes from 3-MC-induced rats effected a seven-fold increase in the formation of AF-M1, while microsomes from PB-induced rats increased the metabolism of AF-B1 to AF-Q1 approximately 2·5-fold. The levels of AF-Q1 formed in the PBB and PCB groups were increased over control levels but did not reach the amounts formed with PB-induced microsomal preparations. Microsomes from PCB- and PBB-induced rats gave 24- and 12-fold increases, respectively, in the conversion of AF-B1 to AF-M1 compared to control microsomes. Figure 2 depicts representative h.p.l.c. scans of metabolite patterns from all five treatment groups. The only aflatoxins detected were AF-B1, AF-Q1 and AF-M1. Other metabolites, when present at a detectable level, elute between AF-Q1 and AF-M1.

The results of pretreatment with PBB and PCB on the metabolism *in vitro* of AF-B1 by kidney microsomes isolated from male rats is shown in table 2. Kidney microsomes from PCB- and PBB-pretreated rats metabolized AF-B1 at rates approximately 10- to 13-fold less than hepatic microsomes from the same animals. Induction by PBB and PCB also stimulated metabolism of AF-B1 to AF-Q1 and AF-M1 but at rates 10- to 30-fold less than those produced by liver microsomes. The results of the metabolism *in vitro* of AF-B1 by microsomes isolated from liver, kidney and mammary tissues, taken from control and PCB-treated lactating female rats are shown in table 3. Although the rates of aflatoxin metabolism were similar, liver microsomes from the female control rats produced lower levels of Q1 and M1 when compared with liver microsomes from male control rats (table 1). Kidney and mammary microsomes from female control rats produced no detectable metabolites. In the rats pretreated with PCBs the liver microsomes metabolized AF-B1 at about four times the rate as the corresponding control microsomes. The PCB-induced kidney and mammary microsomes metabolized AF-B1 at rates approximately six and three times greater, respectively, than control microsomes. The increased metabolism of AF-B1 by microsomes from the PCB-pretreated rats correspond to increases seen in the production of AF-Q1 and AF-M1 as listed in table 3.

Effect of PCB pretreatment on aflatoxin M1 levels in milk

Preliminary experiments with untreated lactating rats showed that daily milk collections of approximately 0·5 ml could be made with no adverse effects to the mother or pups. It was necessary that the pups be returned to the dam between

Table 1. Metabolic activity of hepatic microsomal preparations from male rats treated with either polybrominated biphenyls (PBB), or polychlorinated biphenyls (PCB).

Treatment group (N)	Aflatoxin B1 metabolized		Aflatoxin metabolite				Aminopyrine N-demethylase		Benzo[a]pyrene hydroxylase	
	Rate	Activity as % of control	AF-Q1 formed	Activity as % of control	AF-M1 formed	Activity as % of control	nmol HCHO formed /mg microsomal protein per min	Activity as % of control	nmol BP metabolized/mg microsomal protein per min	Activity as % of control
Corn oil (4)	2·5±1·1	100	0·16±0·02	100	0·5±0·2	100	9·5±0·2	100	0·43±0·01	100
PB (2)	6·2±1·2	248	0·48±0·08*	343	0·3±0·2	55	10·9±0·3	114	0·24±0·01	54
MC (2)	11·7±2.6*	468	0·14±0·12	94	4·2±0·8**	768	9·6±0·3	100	3·10±0·06	725
PBB (4)	21·9±3·5**	876	0·28±0·04*	176	6·7±1·0**	1240	11·0±0·6*	116	1·59±0·10*	361
PCB (4)	42·9±1·3**	1716	0·31±0·19*	197	13·0±0·8**	2400	11·2±0·7*	117	2·20±0·05**	505

The rate of aflatoxin metabolism is expressed as nmol aflatoxin/25 mg microsomal protein per 30 min. The rates of aflatoxin Q1 and M1 formation are expressed as nmol formed/25 mg microsomal protein per 30 min. Values are means ±S.D. for (*N*) animals. The concentration of aflatoxin was 64 nmol/5 ml incubation mixture.

Values statistically different from controls are: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}02$.

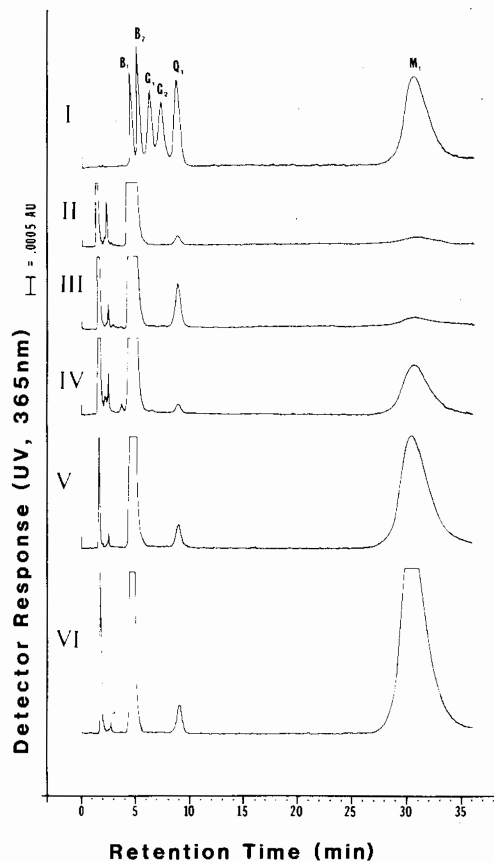

Figure 2. Representative high-pressure liquid chromatograms of chloroform extracts from incubation mixtures consisting of aflatoxin B1 and microsomal preparations from rats pretreated with either phenobarbital, 3-methylcholanthrene, polybrominated biphenyls or polychlorinated biphenyls.

H.p.l.c. scans represent: I, aflatoxin standards (B1, 20 ng; B2, 24 ng; G1, 28 ng; G2, 24 ng; Q1, 50 ng; M1, 200 ng); II, corn oil; III, phenobarbital; IV, 3-methylcholanthrene; V, PBB; VI, PCB. Microsomal incubation mixtures were extracted with chloroform and analysed for aflatoxins using normal phase, radial compression separation with a mobile phase of water-saturated chloroform–cyclohexane–acetonitrile–2-propanol (75:22·5:2·5:3, by vol.); flow rate, 2·0 ml/min; detection, u.v. absorption at 365 nm; temp, ambient; sensitivity, 0·005 AUFS.

Table 2. Metabolism of aflatoxin B1 by male rat kidney microsomes.

Treatment group (N)	Aflatoxin B1 metabolized (nmol metabolized/25 mg microsomal protein per 30 min)	Aflatoxin metabolites (nmol formed/25 mg microsomal protein per 30 min)	
		AF-Q1	AF-M1
Corn oil (4)	3·1 ± 2·4	N.D.	N.D.
PBB (4)	2·6 ± 6·0	0·05 ± 0·01	0·54 ± 0·27
PCB (4)	3·6 ± 5·2	0·02 ± 0·02	0·41 ± 0·34

The concentration of aflatoxin was 64 nmol/5 ml incubation mixture. Values are the means ± S.D. for (N) animals.

N.D. indicates metabolite not detected; limit of detection 0·01 nmol for AF-Q1 and 0·09 nmol for AF-M1.

Table 3. Metabolism of aflatoxin B1 by liver, kidney and mammary microsomes from lactating female rats.

Treatment group (2 rats/group)	Tissue	Aflatoxin (nmol metabolized/ 25 mg microsomal protein per 30 min)	Aflatoxin metabolites (nmol formed/25 mg microcosmal protein per 30 min)	
			AF-Q1	AF-M1
Control				
	Liver	3·1±0·2	0·04±0·01	0·15±0·01
	Kidney	0·3±0·5	N.D.	N.D.
	Mammary	1·9±0·5	N.D.	N.D.
PCB				
	Liver	13·3±2·0	0·17±0·05	1·89±0·22
	Kidney	6·6±1·1	0·17±0·05	0·96±0·55
	Mammary	7·2±1·1	0·09±0·05	0·56±0·03

Microsomes were isolated from female rats 16 day postpartum. The concentration of aflatoxin B1 was 64 nmol/5 ml incubation mixture. Values are means ± S.D. for (N) animals.
N.D. indicates not detected; limit of detection 0·01 nmol for AF-Q1 and 0·09 for AF-M1.

collections to prevent decreases in milk volumes obtained. Multiple collections could be made on the same day but collections on the following day were greatly decreased in volume.

Pretreatment with PCBs had no effect on the volume of milk that could be collected on days 2 and 4 (data not shown). In both control and PCB-treated rats, more than 0·5 ml milk could be obtained after 15 min of milking. There was a significant increase (92%) in the level of aflatoxins excreted in the PCB treatment group (8·3 ± 1·3 nCi/ml, range 5·4–12·1) over that seen in the control group (4·5 ± 0·4 nCi/ml, range 3·8–5·6). H.p.l.c. analysis of milk samples demonstrated only two peaks, identified as AF-B1 and AF-M1. The ratios of AF-M1 to AF-B1 are shown in table 4. In the control group AF-B1 accounted for approximately 30% of the total aflatoxins, while in the PCB-pretreated group this percentage was decreased to approximately 10%.

Discussion

The results from analysis *in vitro* indicated that both PCBs and PBBs are potent inducers of the microsomal enzyme-mediated metabolism of AF-B1. Studies by Gurtoo and Dahms (1979) have reported that pretreatment of rats with 3-MC and PB produces mixed-function oxidases which preferentially metabolize AF-B1 to AF-M1 and AF-Q1, respectively. Both PCB and PBB mixtures have

Table 4. High-pressure liquid chromatographic analysis of aflatoxins in rat milk.

Statistic	Treatment: Control	Treatment: PCB
Aflatoxin B1	2·4±0·9 (1·1–3·5)	0·7±0·8* (0·2–2·0)
Aflatoxin M1	5·9±2·7 (3·6–9·8)	7·7±3·7 (3·7–11·9)
Ratio of aflatoxin M1/B1	2·5	10·24

Levels in nmol/ml milk. Milk samples were taken one hour post-dosing with 0·5 mg/kg aflatoxin B1.
Values significantly different from control group are: *$P < 0·05$.

been classified as 'mixed type' inducers equivalent to the co-administration of PB plus MC (Alvares and Kappas 1977, Dent *et al.* 1977 b).

An approximate four-fold increase in the V_{max} for AF-M1 production was observed with cytochrome P-450 isozymes isolated from PCB-pretreated rats over that seen with P-450 isozymes taken from 3-MC-treated animals (Yoshizawa *et al.* 1982). The rate of AF-Q1 formation was also increased in incubations using cytochrome P-450 isozymes from PCB-treated animals. As expected, microsomes from PBB- and PCB-treated rats stimulated the metabolism of AF-B1 to both AF-M1 and AF-Q1.

In this study the metabolism *in vitro* of AF-B1 to AF-M1 was compared with two other cytochrome P-450-dependent mono-oxygenases, dimethylaminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase (AHH). The *N*-demethylase is a PB-inducible enzyme whereas AHH is primarily induced by 3-MC and related compounds. The PB-, PBB- and PCB-induced microsomes exhibited similar *N*-demethylase activities, however a similar increase in the formation of AF-Q1 was not observed. This difference between the relative levels of AF-Q1 formed and *N*-demethylase activity can best be explained as differences in the forms of cytochrome P-450 induced by PB, PBBs and PCBs. The levels of AF-M1 produced by microsomes from 3-MC-, PBB- and PCB-pretreated rats also showed significant differences when compared to the induction of AHH activity. The difference in AF-M1 formation and AHH activity can be explained as differences in the form of cytochrome P-448 induced. Studies by Gurtoo and co-workers (1978) indicate that AF-B1 4-hydroxylase and AHH are probably different enzymes. This is supported by evidence for the existence of multiple forms of 3-MC-inducible microsomal cytochromes (Negishi and Nebert 1979).

Liver microsomes from PBB-pretreated rats metabolized AF-B1 at approximately eight times the rate shown by control microsomes; whereas, microsomes from PCB-treated rats were considerably more active and metabolized AF-B1 at 17 times the control rate. This is in contrast to previous reports that show that on a weight basis, PBBs are three times more potent than PCBs as inducers of hepatic microsomal (AHH) activities in male rats (Garthoff *et al.* 1977). This is most likely due to differences in the composition of the PCB and PBB mixtures between the two experiments. These chemicals are mixtures of many biphenyl isomers that differ in the amount and orientation of the halide atoms. Differences in the ratios of the various isomers can be significant in terms of what specific form of cytochrome P-450 is induced (Parkinson and Safe 1981).

Our results show that approximately 40% of the aflatoxin metabolized was accounted for by the metabolites AF-Q1 and AF-M1. The remainder of the B1 metabolized is probably involved in binding to microsomal macromolecules and in the formation of minor metabolites.

The data in table 2 demonstrate that male rat kidney microsomes were much less active than liver microsomes in metabolizing AF-B1 to AF-Q1 and AF-M1; however, this activity is readily inducible by PCBs and PBBs. This in turn may play a role in AF-B1-induced tissue-specific toxicity; for example, Akao, Kuroda and Wogan (1971) have shown that in the mouse, a relatively aflatoxin-insensitive species, the principal site of injury following an acute dose was the kidney.

The results in table 3 show that in addition to liver, microsomes from extrahepatic tissues (kidney and mammary) from lactating female rats can be induced by PCBs. The levels of AF-B1 metabolized and metabolites produced by

liver and kidney microsomes, although increased over the corresponding controls, are less than those seen in the male rats (table 1). This may be due to any one of a number of factors including age difference, hormonal differences and stress due to differences in experimental handling and caging.

It has been suggested by Hsieh and co-workers (1977) that the ultimate toxicity of AF-B1 may be determined by competing pathways of activation and detoxification. Thus depending on the type of inducer, the metabolism of the aflatoxins may be shunted into greater, or lesser, toxic metabolites. This is supported by the work of McLean and Marshall (1971) who showed a protective effect of PB pretreatment against AF-B1-induced carcinogenesis and a decrease in the binding of AF-B1 metabolites to DNA *in vitro*. Neal (1976) reported that pretreatment with PB raised the LD_{50} of AF-B1 by 50%, and 3-MC treatment (induction of microsomal cytochrome P-448) decreased the binding of AF-B1 to DNA by 10–60% *in vitro* (Gurtoo *et al.* 1978). It has been shown in the rat, hamster and mouse that the magnitude of AF-B1 binding to nucleic acids correlates well with the susceptibility of each species to AF-B1-induced hepatocarcinogenesis (Ueno, Friedman and Stone 1980). The results of this study have shown that the commercial PCB mixture Aroclor 1254 is a powerful inducer of the conversion of AF-B1 to AF-M1 *in vitro* by microsomal proteins.

It is of public-health interest to assess what effect pretreatment with an inducer such as Aroclor 1254 has on the rate of AF-M1 formation *in vivo* and on the subsequent release into the milk of a lactating animal. The results shown in tables 3 and 4 indicate that the female lactating rat can be used as a model animal in testing the mammary metabolism and excretion of environmental toxins such as AF-B1 and related metabolites. The results in table 4 indicate that pretreatment with PCBs will cause an increase in the amount of AF-M1 released into the milk following dosing with AF-B1. The total aflatoxins (AF-B1 + AF-M1) for the control and PCB groups were approximately the same, yet the ratio of AF-M1 to AF-B1 was four times higher in the PCB-treatment group. Our results indicate that mammary tissue was capable of metabolizing AF-B1 *in vitro*. Thus the diminution in authentic AF-B1 and the increase in AF-M1 levels observed *in vivo* may be due, in part, to mammary metabolism of AF-B1. This in turn may play a role in modulating localized toxicity in the mammary tissue.

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