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Characteristics of Cytochrome P-450 and Mixed Function Oxidase Enzymes Following Treatment with PBBs

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The mixture of PBBs in FireMaster BP-6 has been demonstrated to constitute potent inducers of hepatic and extrahepatic mixed function oxidase (MFO) enzymes. Chronic dietary administration of PBBs to mature female rats results in a "mixed" pattern of induction, with increases in both cytochrome P-450 and P_1-450 associated enzymes. Acute administration of PBBs (150 mg/kg IP) to mature female rats resulted in a time-dependent induction of MFO activities; the P-450-dependent enzymes were simulated early (24-48 hr after administration) while the P_1-450 dependent enzymes reached maximal activities at later time points. However, studies of the kinetics and patterns of inhibition of the induced enzymes along with gel electrophoresis studies of the microsomal proteins indicate that PBBs may induce different proteins from those induced by the classical P-450 and P_1-450 inducers, phenobarbital and 3-methylcholanthrene. In addition, the pattern of enzyme induction caused by PBBs in developing rats differs from that in adults, in that the P_1-450-associated enzymes are stimulated prior to the P-450-associated enzymes. The overall pattern of enzyme induction in extrahepatic tissue differs from that seen in the liver and sex differences in enzyme induction have also been demonstrated. As modifications of MFO activity may alter the toxicity of chemicals, these findings suggest that the toxicity of chemicals may be altered in animals exposed to PBBs and that these toxicities may exhibit age, sex, and organ specificities different from those seen in control animals.

Introduction

The polybrominated biphenyls (PBBs) have, since 1974, become recognized as environmental contaminants, particularly in Michigan (*1*, *2*) and more recently in other states in America (*3*). The PBBs in FireMaster BP-6 (also referred to as FireMaster FF-1 when mixed with an anticaking agent, calcium polysilicate) are a mixture of brominated biphenyls, of which the major components are 2,-2′,4,4′,5,5′-hexabromobiphenyl, which comprises about 60% of the mixture (*4–6*), and 2,2′,4,-4′,5,5′-heptabromobiphenyl, which has been reported to constitute 27% (*7*) or 11% (*8*) by weight. In addition, other bromobiphenyls are present in the mixture (*8*), along with traces of hexa- and pentabrominated naphthalenes and a methyl polybrominated furan (*2*).

Early studies of the toxicity of octabromobiphenyl indicated low acute toxicity but concluded that this compound was biologically persistent (*9*, *10*). Similarly, FireMaster BP-6 appeared to have low acute toxicity and a long biological half-life (*8*, *11*). Because of the chemical similarities between polychlorinated biphenyls (PCBs) and PBBs, it was predicted that the PBBs would have similar biological properties to the PCBs. One of the most potent and thoroughly investigated properties of PCBs is their effects on mixed function oxidase activity (*12–15*).

The mixed function oxidase system (MFO) is located in the endoplasmic reticulum of many mammalian cells (*16–18*). This enzyme system, which has as its terminal oxidase cytochrome P-450, is responsible for the metabolism of many xenobiotics and endogenous compounds (*19*). Many chemicals—drugs, pesticides, herbicides and environmental contaminants—cause alterations in the activity of the MFO system by induction of cytochrome P-450 and the enzyme activities associated with it (*17*). There are several types of cytochrome P-450 inducing agents, but in the main they fall into two distinct classes (*20*, *21*). The first class,

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exemplified by phenobarbital (PB), induces cytochrome P-450 and a wide range of enzyme activities. The second class is exemplified by 3-methylcholanthrene (3MC) which induces a more specific range of enzyme activities and cytochrome P_1-450 (P-448), a cytochrome which differs in several of its physical and chemical properties from the cytochrome P-450 found in control or PB-treated animals (*20, 21*).

It was first reported in 1973 that a commercial mixture of PCBs, Aroclor 1254, shared the inducing properties of both PB and 3MC (*12*). Aroclor 1254 is a mixture of PCBs containing 54% by weight chlorine. The major portion of this mixture is hexachlorobiphenyl. Because of the similarities between PCBs and PBBs, it was predicted that FireMaster BP-6 might similarly be a "mixed" inducer of the MFO system.

To date, the effects of the mixture of PBBs in FireMaster BP-6 on hepatic and extrahepatic MFO activity in neonatal and adult rodents have been investigated. In addition, several studies of the effects of purified components of the FireMaster BP-6 have been reported. This paper reviews these data and discusses the toxicological consequences of these findings.

Effects of PBBs on Hepatic Mixed Function Oxidase

Preliminary studies on FireMaster BP-6 supported the contention that this mixture caused stimulation of hepatic MFO systems (*22*). The pattern of enzyme induction resulting from exposure to PBBs was characterized in a two-week feeding study. Female rats were fed a diet containing 4.96 to 300 ppm FireMaster BP-6, and the profile of hepatic MFO activity which resulted was compared with that observed after a maximal inducing dose of PB or 3MC (*23*). The results from this study clearly indicated that the properties of the PBB mixture were characteristic of a "mixed" inducer. After 2 weeks exposure to 75 and 300 ppm, ethylmorphine-*N*-demethylase activity was elevated to approximately the same extent as seen after a maximal inducing dose of PB; 3MC treatment did not increase this enzyme activity. 3-Methylcholanthrene caused an elevation of ethoxycoumarin-*O*-deethylase and aryl hydrocarbon hydroxylase (AHH) to 40 and 35 times control activity, respectively. Phenobarbital had a much smaller effect on both these enzymes, causing a 9- and 5-fold increase, respectively. At 75 and 300 ppm, PBBs had an effect intermediate between that of PB and 3MC on these enzymes (*23*). In keeping with these observations, a blue shift in the λ_{max} of the difference spectrum of the reduced cytochrome P-450–CO complex was observed in microsomes from PBB-treated animals. The Soret region absorption maxima for the reduced cytochrome P-450–CO complex in control and PB-treated rats is normally close to 450 nm. Treatment of animals with 3MC results in a blue shift of this maximum by about 2 nm to 448 nm (*24*).

From data obtained in the study described above (*23*), it was estimated that the dose of PBBs required to produce maximal induction was between 75 and 300 mg/kg in female rats. In a second study (*25*) a single injection of PBBs was administered intraperitoneally to female rats at 25 or 150 mg/kg, and the time course and pattern of induction studied. Both doses of PBBs resulted in increases in hepatic MFO enzymes sensitive to either PB or 3MC. However, the time at which these enzyme activities reached maximal values differed. The P-450 associated enzymes, those normally sensitive to phenobarbital, were maximally increased at 48 hr after 150 mg/kg. The P_1-450-associated enzymes (3MC-sensitive) reached maximal activities at later times. Similar results were also observed in mice treated with a single dose of PBBs at 150 mg/kg (*26*). The PB-sensitive enzymes were maximally stimulated 48 hr after treatment, and the 3MC-sensitive enzymes 96 hr after treatment with PBBs. In both rats and mice, these changes in enzyme activity were not correlated with changes in cytochrome P-450 content. In both species, the microsomal cytochrome P-450 content increased to maximal levels by 24–48 hr after treatment and then remained elevated and essentially constant for up to 336 hr. However, time-dependent changes in the character of the cytochrome P-450 and some of the associated enzymes were detected.

Several methods have been employed to characterize the type of cytochrome P-450 induced by different compounds. The λ_{max} of the reduced cytochrome P-450–CO complex is altered by P_1-450 inducing agents (*24*). The ratio of the peaks at 455 and 430 nm in the ethyl isocyanide difference spectra is increased by P_1-450 inducing agents, whereas P-450 inducing agents have little or no effect on this ratio (*27*). Enzyme inhibitors have also been used to selectively inhibit P-450 and P_1-450-dependent enzyme activity. Typically, metyrapone preferentially inhibits P-450 mediated AHH activity and α-naphthoflavone inhibits P_1-450-mediated AHH (*28, 29*). In addition, the pattern of protein staining bands between 40,000–60,000 daltons on sodium dodecylsulfate gel electrophoretograms are characteristic of the types of cytochrome(s) P-450 present in microsomes (*15*).

Following the administration of 150 mg/kg PBBs to rats, the maximal shift in the λ_{max} for the reduced cytochrome P-450–CO complex was observed 192 hr after treatment, when the complex absorbed at 448.5 nm compared to 449.1 nm in controls and 448.0 nm in animals treated with a combination of PB and 3MC (*25*). Following five daily injections of PBBs at 90 mg/kg in male rats, the λ_{max} of the reduced P-450–CO complex was observed at 449.5 nm, compared to 448 and 450 nm in microsomes from 3MC- and PB-treated animals, respectively (*7*). In both rats (*25*) and mice (*26*), PBBs cause a change in the ratio of the peaks in the ethyl isocyanide difference spectrum to a value intermediate between those observed in microsomes from PB- and 3MC-treated animals. In addition, time-dependent changes in the peak ratios reflected the time-dependent changes observed in the enzyme activities (*30*).

Changes were also observed in the sensitivity of AHH and ethoxycoumarin-*O*-deethylase activity to the inhibitors metyrapone and α-naphthoflavone (*30*). Following treatment with PBBs, AHH activity became less sensitive to metyrapone while the deethylase activity became more sensitive to metyrapone. Both enzymes were inhibited by α-naphthoflavone to the same extent as seen in control or 3MC-treated animals. No time-dependent change in the sensitivity to α-naphthoflavone could be detected (*30*).

The inhibitor and spectral studies in conjunction with the pattern of enzyme activity induced by PBBs support the contention that the mixture of PBBs in FireMaster BP-6 cause "mixed" induction of cytochrome P-450-dependent enzymes. Furthermore, the pattern of induction observed changes with time after a single injection of PBBs. The changes can be summarized as PB-like (cytochrome P-450) stimulation at early times after treatment followed by a 3MC-like (P_1-450 stimulation) at later times. However, electrophoretic studies of microsomes from PBB-treated rats produced results which are in conflict with this description of the pattern of induction. When microsomes from control and PB-treated animals were submitted to electrophoresis on SDS polyacrylamide gels, the pattern of protein staining bands in the 40,000–60,000 dalton range are very similar. Microsomes from 3MC-treated animals show a distinct band in the region 58,000 daltons which is not evident in microsomes for control of PB-treated animals (*30*), although the purified P_1-450 hemeprotein induced by 3MC is reported to have a molecular weight of about 53,000 daltons (*15*). The studies of the inducing properties of the PBBs described above clearly indicate that they have a 3MC-like component associated with the pattern of enzyme induction they produce. It is therefore surprising that no protein staining band corresponding to the band at 58,000 daltons could be found in microsomes from PBB-treated animals. This observation in conjunction with inhibitor, spectral, and kinetic data led to the suggestion that PBBs may be a different class of inducing agent from the PCBs and led to the speculation that PBBs may not merely act by initiating *de novo* protein synthesis, but may also affect the association of the subunits of cytochrome(s) P-450 in the microsomal membrane (*30*).

However, recent results from Moore et al. (*6, 31*) indicate that in male rats PBBs produce a polypeptide pattern on SDS gel electrophoresis which, judged by both protein and heme staining techniques, resemble the pattern of polypeptides seen after combined PB and 3MC treatment. The reason for these interlaboratory differences is unclear. They may relate to sex or strain differences or they may be a function of the interpretation of the electrophoretic gels. Gel electrophoretograms of whole microsomes are difficult to interpret, especially when using protein stains, due to the large amount of non-cytochrome P-450 related protein, and the presence of other proteins in the 40,000–60,000 dalton range (*32, 33*). Unambiguous identification of the cytochrome P-450 related hemeproteins induced by PBBs awaits purification of the cytochromes involved. Interestingly, in a recent study of purified cytochrome P-450 from rats treated with Aroclor 1254, Ryan et al. (*15*) concluded from spectral, electrophoretic, immunological, and kinetic data that the PCBs did induce cytochrome P-450 hemeproteins which were, in almost every respect, identical to a mixture of PB and 3MC-induced hemeproteins. A similar experimental approach will unambiguously establish the nature of the hemeproteins induced by PBBs.

Inducing Properties of the Components of FireMaster BP-6

The time-dependent changes in MFO activity following administration of PBBs discussed above could be due to differences in the susceptibility of the different enzymes to induction. Alternatively, these time-dependent changes could relate to the pharmacokinetics of the constituents of the PBB mixture. Studies of the rate of absorption of the PBB cogeners from the peritoneal cavity of young rats indicate that there are no detectable differences in the rates of absorption of the major components of the mixture during the first 14 days after an intraperitoneal injection (*34*). This observation does

not preclude the possibility that a minor component of the PBB mixture is more slowly absorbed, and that this component is responsible for the 3MC-like induction. As yet we do not know in detail which components of the mixture are responsible for which of the biochemical effects.

The first of the components of the PBB mixture to be isolated, purified, and investigated biochemically is the major component 2,2',4,4',5,5'-hexabromobiphenyl (*6*). This isomer, which has been purified to better than 99%, clearly has the properties of a PB-type inducing agent. The hexabromobiphenyl isomer increases cytochrome P-450 concentration without changing the λ_{max} for the P-450–CO complex and causes increases in enzyme activity which are almost identical to those resulting from PB treatment (*6*, *31*). Moore et al. have also isolated the second most abundant component in FireMaster BP-6, namely 2,2',3,4,4',5,5'-heptabromobiphenyl (*7*), and a minor component 2,2'-dibromobiphenyl (*31*). Studies of the inducing properties of these two isomers led to the conclusion that the heptabromo isomer has PB-like inducing properties while the dibromo isomer does not have a significant effect on MFO activity (*31*). Although Moore et al. (*6*, *31*) have, as yet, only studied the inducing properties of three pure PBB cogeners, the pattern of induction produced by these pure compounds is similar to those of the corresponding purified PCBs. In an extensive study of the inducing properties of purified PCBs, Goldstein et al. (*14*) concluded that the absence of chlorine atoms in the *ortho* positions of the biphenyl rings was a prerequisite for the isomer to exhibit a 3MC-like inducing effect. They postulate that coplanarity of the biphenyl rings is a prerequisite for a P_1-450 induction; coplanarity is inconsistent with *ortho*-substituted chlorines. They found that 2,2'-dichlorobiphenyl was inactive as an inducer; Moore et al. (*31*) found similar results for the corresponding 2,2'-dibromobiphenyl.

Stimulation of Extrahepatic Mixed Function Oxidase Activity by PBBs

While the liver is recognized as the major organ of xenobiotic metabolism, studies of MFO activity in extrahepatic tissue reveal a diversity of organs with the ability to metabolize xenobiotics (*16–18*). In addition, there is an increased awareness of the potential role of the MFO systems in specific organs in the response of the organs to the toxic action of a chemical. Therefore, the effects of PBBs on extrahepatic MFO activity is of considerable interest.

In the light of reports of detectable levels of PBBs in human breast milk (*11*) and the observation that milk forms a major route of excretion of PBBs in cattle (*35*), the effects of PBBs on mixed function oxidase activity in mammary tissue warrant attention. When pregnant rats were fed 50 ppm PBB from day 8 of gestation until day 14 postpartum, the mammary glands had approximately 30 times higher concentrations of PBBs than the liver. The mammary AHH activity was three times higher in treated animals than in the controls. This elevated AHH activity, although not as great as that observed in the liver, where a 10-fold increase was noted, was accompanied by a decrease in epoxide hydratase activity to half the control activity (*36*). The decrease in epoxide hydratase activity after treatment with PBBs, while unusual, is not confined to mammary tissue. In another series of experiments, male rats were fed 100 ppm PBBs for 3 months. When the renal AHH and epoxide hydratase activities were measured, AHH was elevated to ten times control levels, whereas the epoxide hydratase activity was barely detectable in the PBB-treated animals and represented approximately 10% of control activity (*37*). These effects were not reproduced by a single injection of PBBs in developing rats (*38*). In these studies, 7-day-old rats received a single IP injection of 150 mg/kg PBBs. Renal ethoxyresorufin-*O*-deethylase and AHH activity was increased above control activity by 7 days after treatment and remained elevated 63 days after treatment. In contrast, renal hexobarbital and biphenyl hydroxylase activities could not be detected in control animals, and were not detectable in PBB-treated animals. Renal epoxide hydratase was unchanged by PBB treatment (*38*). McCormack et al. also report the PBBs alone or in combination with PCBs cause dose-related increases in renal, mammary, hepatic, and pulmonary AHH activity (*40*). They conclude that FireMaster BP-6 is more potent, on a weight basis, as an enzyme inducer than Aroclor 1254, in agreement with the results of Garthoff et al. (*22*), and that no synergism was noted between PBBs and PCBs in their inducing properties, although some additive effects of these two mixtures on MFO activity was noted. In contrast, Elcombe and Lech report that PBBs are less potent inducers of hepatic MFO activity in trout than are the PCBs Aroclor 1242 and 1254 (*39*). Their results also suggest that PBBs have different inducing properties from the PCBs.

Effects of PBBs on MFO Activity in Neonatal Animals

It was first reported in early 1976 by Moore et al. that PBBs, when fed to nursing rats at doses as low as 1 ppm in the diet for 18 days postpartum, caused

significant increases in hepatic MFO activity in the suckling pups, but had little effect on hepatic MFO activity in the mother at this dose (*41*). These results are presumably a reflection of the bioconcentration of PBBs in breast milk which leads to the suckling pups receiving a high dose of PBB relative to their body weights. The PBBs do not only cause enzyme induction when transferred to the suckling pup in the breast milk. Circumstantial evidence also exists that transplacental passage of PBBs occurs, although this is not surprising in light of the known ability of the PCBs to cross the placental barrier (*42*). In an experiment designed to distinquish the pre- and postnatal effects of PBBs, pregnant rats were fed diet containing 50 ppm PBBs from day 8 of gestation until day 14 postpartum. At birth, all pups were cross fostered so that they received 0 ppm PBBs or were exposed to PBBs either prenatally, postnatally or both pre- and postnatally. In all pups exposed to PBBs, regardless of the exposure regimen, elevated hepatic AHH and EH activity was detected. Combined pre- and postnatal exposure caused the greatest increases (*43*). It was concluded that PBBs were effective prenatal inducing agents and their effects were assumed to result from transplacental passage of PBBs. This assumption was supported by analytical findings of PBBs in animals born to mothers fed PBBs, but fostered by control animals (*44*).

When the enzyme inducing properties of PBBs were investigated in developing rats by injecting 7-day-old animals with 150 mg/kg (*37*), several interesting phenomena were observed. Firstly, in the liver, 3MC-sensitive P_1-450-dependent enzymes were elevated earlier than the PB-sensitive P-450 dependent enzymes. This presumably reflects the greater sensitivity of the developing rat to 3MC-type inducing agents (*45*). In addition, when MFO activities in the males were compared with those in the females, 28 and 63 days after treatment (the animals were then 35 and 70 days old) a greater incidence of sex differences in enzyme activities was observed as compared to control animals. Furthermore, renal ethoxyresorufin-*O*-deethylase and AHH activities were higher in treated females than in treated males, a reversal of the normal pattern of sex-dependent differences in enzyme activity.

Toxicological Consequences of Mixed Function Oxidase Stimulation by PBBs

In general, the PBBs have a relatively low acute toxicity in mammals. The acute LD_{50} in rats for FireMaster BP-6 is reported to be 21.5 g/kg (*8*). In addition, several studies have reported prolonged exposure of laboratory rodents to PBBs at doses up to 1000 ppm in the diet, without excessive or overt toxic effects (*2*). However, there are indications that the PBBs may be carcinogenic (*46*) and teratogenic (*2*) in rodents at high doses. Considerable effort is now being directed towards identifying the human health hazards associated with exposure to PBBs.

How do the studies reviewed here relate to the potential human health hazard associated with exposure to PBBs, and how may they be used to assist in further investigations in this area?

Enzyme inducing agents have been shown to modify the toxicity of chemicals (*44–49*). Both PB and 3MC modify the toxicity of agents such as CCl_4, acetaminophen, and bromobenzene. The PCB Aroclor 1254 has been shown to increase the toxicity of CCl_4 (*50*). These changes in toxicity relate to changes in the pattern or rate of metabolism of the toxic agents. Different classes of inducing agents have different effects on the toxicity of secondary compounds. For example, pretreatment of animals with PB enhances the hepatotoxicity of bromobenzene while 3MC-pretreated animals are protected from the hepatotoxicity of this agent (*51*). As the inducing properties of PBBs change with time, after a single dose, from PB-like at early times to 3MC-like at later times after administration (*25, 26*) it was predicted that PBBs would modify the toxicity of bromobenzene in a time dependent fashion. This proved to be the case. At early times after PBB administration, the toxicity of bromobenzene was modified in a PB-like manner; at later times after administration, PBBs had a 3MC-like effect on bromobenzene toxicity (*52*).

The observed age, sex, and organ specific responses of the MFO system to PBBs suggest that the toxicity of secondary agents in PBB exposed animals may be age, sex and organ dependent. The depression of epoxide hydratase in kidney and mammary tissue implies that the ability of these organs to convert epoxides to dihydrodiols may be compromised. In light of the postulated role of epoxides in the toxicity of alkene and arene compounds (*53*) the consequences of these biochemical changes elicited by PBB exposure may be far reaching. The hepatotoxicity and nephrotoxicity of chloroform is enhanced by prior exposure of mice to PBBs (*54*). In addition, changes in the MFO system in the mammary gland could have nutritional and toxicological consequences for the suckling young. In recent years, the role of milk in the transfer of foreign compounds from mother to young has been recognized (*54*). We may speculate

that changes in MFO activity in the mammary gland may alter both the quantity and chemical identity of compounds transferred from mother to young in the milk.

The observed accumulation of PBB in the testes and ovaries of rats exposed to PBB (*34*) may be a reflection of the high fat content of these organs. However, the biological consequences of this accumulation have not been investigated. Whether PBBs modify MFO activity in the sex organs remains to be determined. Changes in sexual function have been reported in animals exposed to PCBs (*56*). Whether similar changes occur in PBB exposed animals has not been determined. Conceivably modification of MFO activity in sex organs could alter the metabolism of sex hormones in these organs as well as any effect that may occur in the liver. The PCBs have been shown to modify metabolism of thyroxine in rats (*57*). Mixed function oxidase inducers are known to modify the metabolism of many endogenous and exogenous compounds in man including the sex hormones (*58*).

In conclusion, a recent report (*45*) estimated that human exposure to PBBs may in some cases be as much as 200 mg/kg. While this estimate may be high, the animal studies reviewed here indicate that low doses of PBBs in rodents have a significant effect on MFO activity. These observations, combined with the long biological half-life of the PBBs and their propensity to accumulate in the body, raise concern as to the effects of PBBs on MFO activity in the exposed human population. Clearly, the potential health effects of altered MFO activity warrants consideration in future studies of the human population exposed to polybrominated biphenyls.

REFERENCES

1. Carter, L. J. Michigan's PBB incident: Chemical mix-up leads to disaster. Science 192: 240 (1976).
2. Kay, K. Polybrominated biphenyls (PBB) environmental contamination in Michigan 1973-1976. Environ. Res. 13: 74 (1977).
3. Anonymous. Traces of PBBs found in two states. Chem. Eng. News 55 (26): 16 (1977).
4. Sunström, G., Hutzinger, O., and Safe, S. Identification of 2,2',4,4',5,5'-hexabromobiphenyl as the major component of flame retardant FireMaster BP-6. Chemosphere 1: 11 (1976).
5. Jacobs, L. W., Chou, S. F., and Tiedje, J. M. Fate of polybrominated biphenyls (PBBs) in soils. Persistence and plant uptake. J. Agr. Food Chem. 24: 1198 (1976).
6. Moore, R. W., Sleight, S. D., and Aust, S. D. Induction of liver microsomal drug metabolizing enzymes by 2,2',4,4',5,5'-hexabromobiphenyl. Toxicol. Appl. Pharmacol., in press.
7. Moore, R. W., O'Connor, J. V., and Aust, S. D. Identification of a major component of polybrominated biphenyls a Environ. Tox. Contam., in press.
8. Michigan Chemical Corporation. A report on polybrominated biphenyls prepared for the Michigan Environmental Review Board. September 23, 1974.
9. Lee, K. P., et al. Octobromobiphenyl-induced ultrastructure changes in rat liver. Arch. Environ. Health 30: 465 (1975).
10. Lee, K. P., et al. Bromine tissue residue and hepatoxic effects of octobromobiphenyl in rats. Toxicol. Appl. Pharmacol. 34: 115 (1975).
11. Final Report of the Subcommittee on Health Effects of Polychlorinated Biphenyls and Polybrominated Biphenyls. Department of Health, Education and Welfare, Washington, D. C., July 1976.
12. Alvares, A. P., Bickers, D. R., and Kappas, A. Polychlorinated biphenyls: A new type of inducer of cytochrome P_{448} in the liver. Proc. Nat. Acad. Sci. (U. S.) 70: 1321 (1973).
13. Goldstein, J. A., et al. A comparative study of two polychlorinated biphenyl mixtures (Aroclors 1242 and 1016) containing 42% chlorine on induction of hepatic porphyria and drug metabolizing enzymes. Toxicol. Appl. Pharmacol. 32: 461 (1975).
14. Goldstein, J. A., et al. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem. Biol. Interact. 17: 69 (1977).
15. Ryan, D. E., Thomas, P. E., and Levin, W. Properties of purified liver microsomal cytochrome P450 from rats treated with the polychlorinated biphenyl mixture Arochlor 1254. Mol. Pharmacol. 13: 521 (1977).
16. Gram, T. E. Comparative aspects of mixed function oxidation by lung and liver of rabbits. Drug Metab. Rev. 2: 1 (1973).
17. Parke, D. V. Induction of drug-metabolizing enzymes. In: Enzyme Induction. D. V. Parke, Ed., Plenum Press, London and New York, 1975.
18. Andrews, L. S., Sonawane, B. R., and Yaffe, S. J. Characterization and induction of aryl hydrocarbon (benzo[a]pyrene) hydroxylase in rabbit bone marrow. Res. Comm. Chem. Path. Pharm. 15: 319 (1976).
19. Conney, A. H. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19: 317 (1967).
20. Sladek, N. E., and Mannering, G. J. Induction of drug metabolism. I. Differences in the mechanism by which polycyclic hydrocarbons and phenobarbital produce their inductive effects on microsomal *N*-demethylating systems. Mol. Pharmacol. 5: 174 (1969).
21. Sladek, N. E., and Mannering, G. J. Induction of drug metabolism. II. Qualitative differences in the microsomal *N*-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. Mol. Pharmacol. 5: 186 (1969).
22. Garthoff, L. H., et al. Biochemical and cytogenetic effects caused by short-term ingestion of Aroclor 1254 or FireMaster BP6. J. Toxicol. Environ. Health 3: 769 (1977).
23. Dent, J. G., Netter, K. J., and Gibson, J. E. Effects of chronic administration of polybrominated biphenyls on parameters associated with hepatic drug metabolism. Res. Comm. Chem. Pathol. Pharmacol. 13: 75 (1976).
24. Sladek, N. E., and Mannering, G. J. Evidence for a new P-450 hemoprotein in hepatic microsomes from methylcholanthrene treated rats. Biochem. Biophys. Res. Commun. 23: 668 (1966).
25. Dent, J. G., Netter, K. J., and Gibson, J. E. The induction of hepatic microsomal metabolism in rats following acute administration of a mixture of polybrominated biphenyls. Toxicol. Appl. Pharmacol. 38: 237 (1976).
26. Dent, J. G., et al. Stimulation of hepatic microsomal metabolism in mice by a mixture of polybrominated biphenyls. J. Toxicol. Environ. Health 3: 651 (1977).
27. Imai, Y., and Sato, R. Evidence for two forms of P-450 hemoprotein in microsomal membranes. Biochem. Biophys. Res. Comm. 23: 5 (1966).

28. Wiebel, F. J., and Gelboin, H. V. Aryl hydrocarbon hydroxylase in liver from rats of different ages, sex and nutritional status. Distinction of 2 types by 7, 8 benzoflavone. Biochem. Pharmacol. 24: 1511 (1975).
29. Goujon, F. M., Nebert, D. W., and Gielen, J. E. Genetic expression of arylhydrocarbon hydroxylase induction. IV. Interaction of various compounds with different forms of cytochrome P-450 and effects on benzo[a]pyrene metabolism *in vitro*. Mol. Pharmacol. 8: 667 (1972).
30. Dent, J. G., et al. Rat hepatic microsomal cytochrome(s) P-450 induced by polybrominated biphenyls. Drug Met. Disp. 6: 96 (1977).
31. Dannan, G. A., Moore, R. W., and Aust. S. D. Studies on the microsomal metabolism and binding of polybrominated biphenyls (PBBs). Environ. Health Perspect. 23: 51 (1978).
32. Strittmatter, P., et al. Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. Proc. Natl. Acad. Sci. (U. S.) 71: 4565 (1974).
33. Lu, A. Y. H., et al. Liver microsomal epoxide hydratase. Solubilization, purification, and characterization. J. Biol. Chem. 250: 8283 (1975).
34. Rickert, D. E., et al. Distribution of polybrominated biphenyls in the developing rat following a single intraperitoneal injection. Drug Met. Disp., submitted.
35. Fries, G. E., and Marrow, G. S. Excretion of polybrominated biphenyls into the milk of cows. J. Dairy Sci. 58: 947 (1975).
36. Dent, J. G., et al. Liver and mammary arylhydrocarbon hydroxylase and epoxide hydratase in lactating rats fed polybrominated biphenyls. Life Sci. 20: 2075 (1977).
37. McCormack, K. M., et al. Effects of polybrominated biphenyls on kidney function and the activity of renal microsomal enzymes. Environ. Health Perspect. 23: 153 (1978).
38. McCormack, K. M., et al. Stimulation of hepatic and renal mixed function oxidase in developing rats by polybrominated biphenyls. Drug Met. Disp., submitted.
39. Elcombe, C. E., and Lech, J. J. Induction of monooxygenation in rainbow trout by polybrominated biphenyl: a comparative study. Environ. Health Perspect. 23: 309 (1978).
40. McCormack, K. M., et al. Concomitant dietary exposure to polychlorinated biphenyls and polybrominated biphenyls; Tissue distribution and arylhydrocarbon hydroxylase activity in lactating rats. Toxicol. Appl. Pharmacol., submitted.
41. Moore, R. W., Ghazi, D., and Aust, S. D. Induction of drug metabolizing enzymes in rats nursing from mothers fed polybrominated biphenyls. Fed. Proc. 35: 706 (1976).
42. Grant, D. L., et al. Placental transfer of polychlorinated biphenyls in the rabbit. Environ. Physiol. 1: 61 (1971).
43. Dent, J. G., et al. Mixed function oxidase activities in lactating rats and their offspring following dietary exposure of polybrominated biphenyls. Toxicol. Appl. Pharmacol., submitted.
44. Rickert, D. E., et al. Distribution of polybrominated biphenyls after dietary exposure in pregnant and lactating rats and their offspring. Environ. Health Perspect. 23: 63 (1978).
45. Dickerson, J. W. T., and Basu, T. K. Enzyme induction in the process of development. In: Enzyme Induction, D. V. Parke, Ed., Plenum Press, London and New York, 1975.
46. Kimbrough, R. D., Burse, V. M., and Liddle, J. A. Toxicity of polybrominated biphenyl. Lancet 602 (1977).
47. Conney, A. H., and Burns, J. J. Metabolic interactions among environmental chemicals and drugs. Science 178: 576 (1972).
48. Gillette, J. R. Environmental factors in drug metabolism. Fed. Proc. 35: 1142 (1976).
49. Mitchell, J. R., and Jollow, D. J. Metabolic activation of drugs to toxic substances. Gastroenterol. 68: 392 (1975).
50. Carlson, G. P. Potentiation of carbon tetrachloride hepatotoxicity in rats by pretreatment with polychlorinated biphenyls. Toxicology 5: 69 (1975).
51. Reid, W. D., et al. 3-Methylcholanthrene blocks hepatic necrosis induced by administration of bromobenzene or carbon tetrachloride. Exptl. Mol. Pathol. 15: 363 (1971).
52. Roes, U., et al. Effect of polybrominated biphenyls on bromobenzene lethality in mice. J. Toxicol. Environ. Health 3: 663 (1977).
53. Daly, J. W., Jerina, D. M., and Witkop, B. Arene oxides and the NIH shift: The metabolism, toxicity and carcinogenicity of aromatic compounds. Experientia 28: 1129 (1972).
54. Kluwe, W. M., et al. Potentiation of hepatic and renal toxicity of various compounds by prior exposure to polybrominated biphenyls. Environ. Health Perspect. 23: 241 (1978).
55. Knowles, J. A. Breast milk: a source of more than nutrition for the neonate. Clin. Toxicol. 7: 69 (1974).
56. Kihlström, J. E., et al. Sexual function of mice neonatally exposed to DDT or PCB. Environ. Physiol. Biochem. 5: 54 (1975).
57. Bastomsky, C. H., Murthy, P. V. N., and Banovac, K. Alterations in thyroxine metabolism produced by cutaneous application of microscope immersion oil: Effects due to polychlorinated biphenyls. Endocrinol. 98: 1309 (1976).
58. Breckenridge, A. Clinical implications of enzyme induction. In: Enzyme Induction, D. V. Parke, Ed., Plenum Press, London and New York, 1975.