

# Induction of Drug Metabolizing Enzymes in Polybrominated Biphenyl-Fed Lactating Rats and Their Pups

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Polybrominated biphenyls (PBBs) cause a mixed-type (phenobarbital- plus 3-methylcholanthrene-like) induction of liver microsomal drug metabolizing enzymes in rats. However, 2,2',4,4',5,5'-hexabromobiphenyl and 2,2',3,4,4',5,5'-heptabromobiphenyl, which together comprise >80% of PBBs (FireMaster), were shown to be strictly phenobarbital-type inducers. Other components (unidentified) must therefore cause the 3-methylcholanthrene-like effects.

The potential for PBBs to exert effects on neonates through milk was examined. Lactating rats were fed 0, 0.1, 1.0, or 10 ppm FireMaster for the 18 days following delivery, at which time mothers and most pups were sacrificed. Pups nursing from mothers fed 10 ppm PBBs showed significant increases in liver weights and microsomal protein, and both mothers and pups had increased cytochrome P-450, aminopyrine demethylation, benzo[a]pyrene hydroxylation, and UDP-glucuronyltransferase. Pups nursing from rats fed 1.0 ppm had increases in microsomal protein, cytochrome P-450, aminopyrine demethylation, and benzo[a]pyrene hydroxylation, while their mothers were unaffected. Several pups from the 0, 0.1, and 1.0 ppm groups were maintained on their mothers' diets, raised, and allowed to mate. Their pups showed much the same responses to PBBs as did the original group of pups. The effects on both generations of adult female rats were also comparable.

PBBs cause a mixed-type induction in both lactating rats and their nursing pups; PBB components responsible for both aspects of this induction must be transmitted through milk. Nursing rats are approximately tenfold more sensitive to the effects of PBBs in their mothers' diets than are the dams. The approximate no-effect level for microsomal induction in nursing rats is 0.1 ppm PBBs in the diet of the adult.

## Introduction

Polybrominated biphenyls (PBBs) are known to cause a mixed-type induction of rat liver microsomal drug metabolizing enzymes. These induced microsomes display the properties seen in response to both phenobarbital (Pb) and to 3-methylcholanthrene (MC) (1-7). A review by Dent of the effects of PBBs on drug metabolizing enzymes is included in this volume (8). PBBs, however, are a complex mixture of several dozen components (3), including brominated naphthalenes (9), and an understanding of which of these components induce

microsomal enzymes, and of the nature of the induction caused by the individual components, is only now beginning to emerge.

At the Symposium on PBBs, we presented the results of several experiments which determined the effects of purified PBB components on the drug metabolizing enzymes. The major component of FireMaster, 2,2',4,4',5,5'-hexabromobiphenyl (HBB<sub>6</sub>), comprises 56% by weight of this mixture (3). HBB<sub>6</sub> was shown to be strictly a Pb-type inducer of microsomal enzymes, by analysis of the enzymatic activities, spectral properties, and polyacrylamide gel electrophoretic properties of the induced microsomes (3). A full description of these experiments will have been published by the time these proceedings appear (3); to avoid duplication, the detailed results will not be presented in this paper. We also presented data concerning the ef-

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fects of 2,2'-dibromobiphenyl and 2,2',3,4,4',5,5'-heptabromobiphenyl (HBB<sub>7</sub>) on liver microsomal drug metabolizing enzymes. The dibromobiphenyl, a suspected trace component of PBBs, was shown to have virtually no effect on any parameter examined, even though the rats were given a 90 mg/kg IP injection (4). We have shown this compound to be rapidly metabolized *in vitro* by liver microsomes (10), and so it appears that neither the parent molecule nor its metabolites were effective inducers. The other congener tested for its inducing effects, HBB<sub>7</sub>, comprises 27% by weight (11) of the FireMaster (lot 7042) (12) which contaminated much of the Michigan food chain beginning in 1973 (13). By using the same criteria as were applied to evaluating the effects of HBB<sub>6</sub>, HBB<sub>7</sub> was found to be only a Pb-type inducer (4). Neither HBB<sub>6</sub> nor HBB<sub>7</sub> showed any evidence of being metabolized *in vitro* by microsomes (10). The detailed results describing the effects of 2,2'-dibromobiphenyl and HBB<sub>7</sub> will be published elsewhere (4).

As described above, the two major congeners, which comprise greater than 80% by weight of PBBs (FireMaster) are strictly Pb-type inducers. The effects of the remaining several dozen PBB components on drug metabolizing enzymes or any other biological parameters have not been evaluated. Indeed, only a relatively small number of these components have even been structurally identified (11, 14-16). Although it had previously been documented that PBB components could be secreted in milk (17, 18), in the absence of knowledge as to their biological effects, this information could not be used to fully predict the effects of their secretion on nursing animals. One objective of the research presented in this paper was to determine whether those components responsible for inducing the full range of drug metabolizing enzymes could be transmitted through milk from lactating rats to their offspring. It was also of considerable interest to determine a dietary limit for the lactating animals below which the drug metabolizing enzymes in their nursing pups would not be effected. A preliminary report of this research has been published (19).

## Methods

### Animals

Studies of the effects of purified PBB components were done on male rats weighing approximately 170 g. The rats were given a single IP injection, at 90 mg/kg body weight, then sacrificed at intervals up to 22 days later. This quantity of injected material had been shown to cause a maximal induction in re-

sponse to both HBB<sub>6</sub> and PBBs (3). The liver microsomes from these animals were compared with microsomes isolated from rats pretreated with maximally effective doses of MC, Pb, and PBBs. Full details of these procedures may be found elsewhere (3, 4).

For the experiments with lactating and nursing rats, pregnant female Sprague-Dawley rats were purchased from Spartan Research Animals, Inc., Haslett, Michigan. One the day of delivery, the mothers' diet of pelleted feed was replaced with ground rat chow containing 2 ml/kg corn oil and 0, 0.1, 1.0, or 10 ppm PBBs (FireMaster FF-1, lot 7042). Care was taken to ensure that pups did not have access to the feed. Eighteen days after delivery, the mothers and most of their pups were sacrificed by decapitation. The night before sacrifice, feed was removed from the cages, but pups were allowed to continue nursing. Liver microsomes were isolated and washed individually from each dam and from most of her pooled pups (3). There were four animals in each group, however, one pooled liver sample from the 1.0 ppm pups was lost during homogenization.

Six pups apiece from the control, 0.1, and 1.0 ppm groups were saved and placed on the diets their mothers had been consuming. These rats were raised and allowed to mate. Eighteen days after delivery, they and their pups were sacrificed, and liver microsomes were isolated and washed as described (3).

### Enzyme Assays

NADPH-cytochrome P-450 reductase was assayed by its ability to reduce cytochrome c (20), and cytochrome P-450 was assayed spectrally in the presence of 10% glycerol (21). Aminopyrine demethylation was assayed by monitoring formaldehyde production (3), and benzo[a]pyrene hydroxylation was determined fluorimetrically (22). UDP-glucuronyltransferase was measured by using *p*-nitrophenol as the acceptor (3). Protein was assayed by the method of Lowry et al. (23), standardized with bovine serum albumin as described by Rutter (24).

### Statistical Analysis

Statistical analysis of the data was by Student's *t* test, with *p* < 0.05 considered to be significant.

## Results

HBB<sub>6</sub> and HBB<sub>7</sub> caused large increases in liver weights and microsomal protein. They also strongly

induced NADPH-cytochrome P-450 reductase, cytochrome P-450, aminopyrine demethylation, and epoxide hydratase. In contrast, they proved to be only weak inducers of benzo[a]pyrene hydroxylation and UDP-glucuronyltransferase, and failed to shift the cytochrome P-450 spectral maximum from 450 nm. SDS-polyacrylamide gel electrophoresis of these microsomes showed that they were virtually identical to microsomes induced by Pb, when both the heme and protein profiles were examined. In all respects, the effects of HBB<sub>6</sub> and HBB<sub>7</sub> were very similar to those observed when rats were treated with Pb, but PBBs had several distinctly different effects. Together, these components comprise 83% by weight of the PBB sample studied which was shown to be a mixed-type inducer. Complete descriptions of these results are being published elsewhere (3, 4).

Results of the studies with PBBs (FireMaster FF-1) on lactating and nursing rats are given below.

Dietary PBBs had no effect on the liver weights of the mothers, but increased liver weight in the pups (Fig. 1). There was a nonsignificant 22% increase at 1.0 ppm, and a highly significant 39% increase in pups whose mothers were fed 10 ppm PBBs.

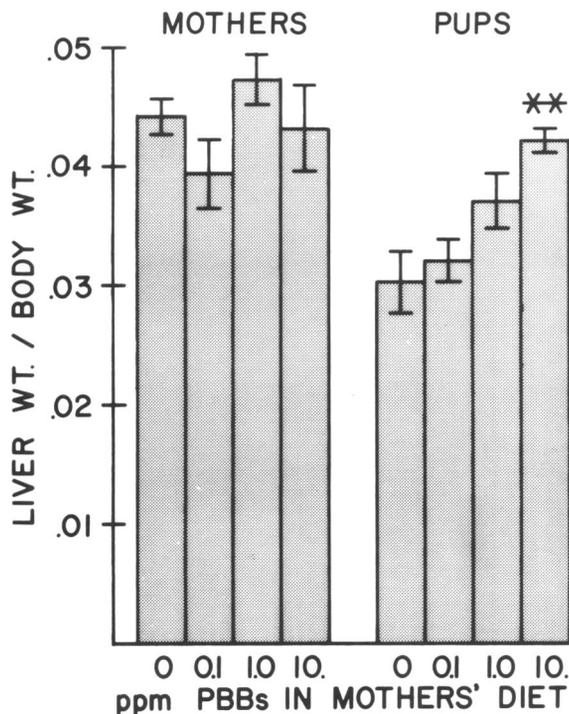


FIGURE 1. Effects of PBBs in the maternal diet on liver weights in dams and pups. Exposure was for the eighteen days following delivery. Values are means  $\pm$  SEM,  $N=4$ . Values significantly different from corresponding controls at  $p < 0.05$  and  $p < 0.01$  are indicated by \* and \*\*, respectively.

There appeared to be a slight trend towards increasing liver microsomal protein in the lactating rats, but the differences were not significant. The pups showed significant increases at both 1.0 and 10 ppm PBBs, the latter causing a 94% increase (Fig. 2).

NADPH-cytochrome P-450 reductase appears to have been only slightly induced by PBBs in both mothers and pups (Fig. 3). In contrast, cytochrome P-450 was induced 64% in rats fed 10 ppm PBBs, and their pups were induced by 210% over the control values. While dams fed 0.1 or 1.0 ppm PBBs showed no induction, pups nursing from the 1.0 ppm mothers also had significantly elevated concentrations of cytochrome P-450 (Fig. 4). In all cases,  $\lambda_{max}$  in the spectrum was at 450 nm.

Aminopyrine demethylation was assayed as a measure of the extent of Pb-type induction of the liver mixed-function oxidases (25). While dams were induced 58% by consuming 10 ppm PBBs, their pups were induced 190%, and pups nursing from mothers fed 1.0 ppm PBBs also showed a 78% induction, while their mothers were unaffected (Fig. 5). Benzo[a]pyrene hydroxylation was likewise assayed as a measure of the MC-like aspects of the induction (26). Mothers fed 10 ppm PBBs and pups nursing from mothers fed both 1.0 and 10 ppm PBBs were all very strongly induced (Fig. 6).

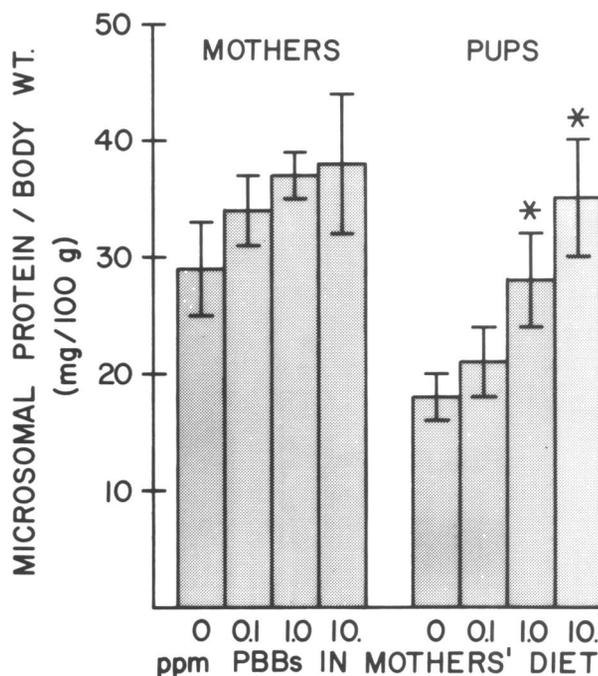


FIGURE 2. Effects of PBBs in the maternal diet on microsomal protein in dams and pups. Details as in Figure 1.

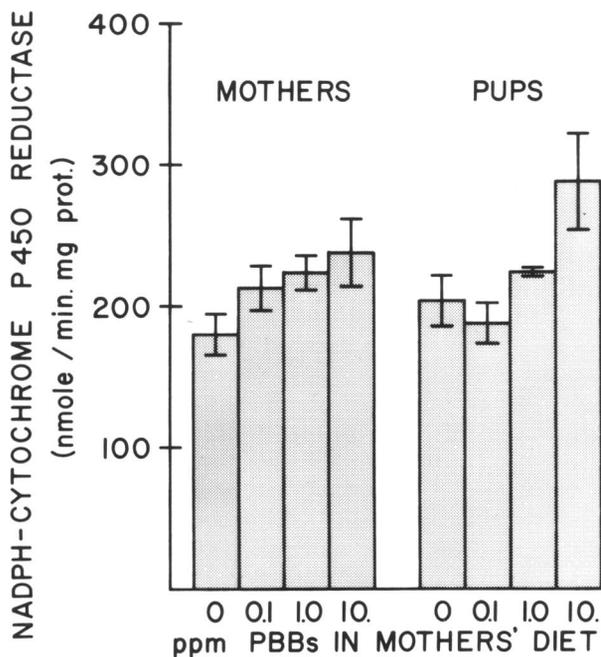


FIGURE 3. Effects of PBBs in the maternal diet on NADPH-cytochrome P-450 reductase in dams and pups. Details as in Figure 1.

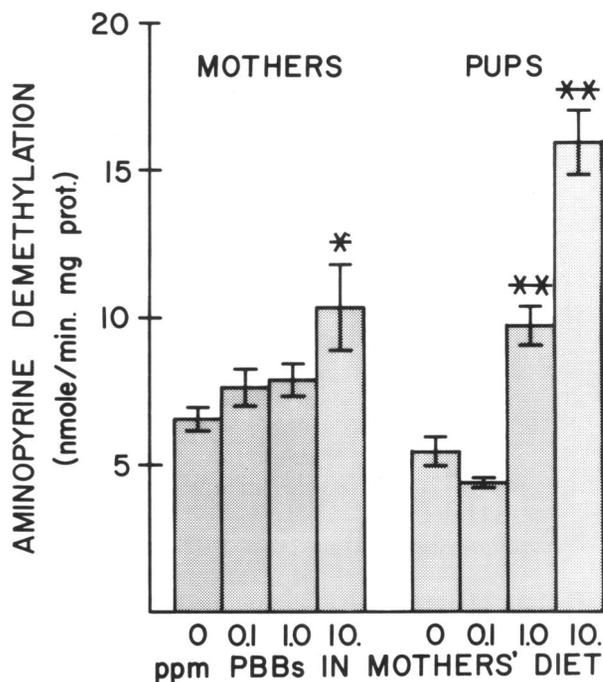


FIGURE 5. Effects of PBBs in the maternal diet on aminopyrine demethylation in dams and pups. Details as in Figure 1.

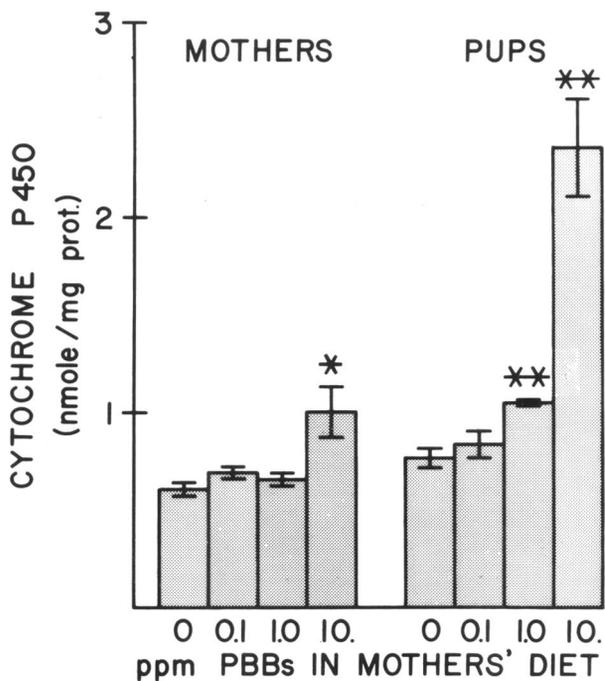


FIGURE 4. Effects of PBBs in the maternal diet on cytochrome P-450 in dams and pups. Details as in Figure 1.

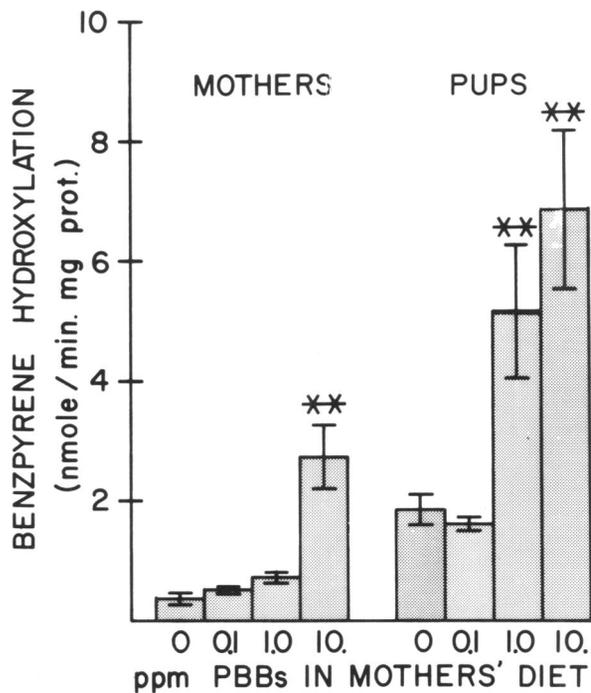


FIGURE 6. Effects of PBBs in the maternal diet on benzo[a]pyrene hydroxylation in dams and pups. Details as in Figure 1.

UDP-glucuronyltransferase was assayed as an index of the ability of microsomes to conjugate hydroxylated substrates, perhaps including metabolites of PBB congeners. The acceptor used was *p*-nitrophenol, which measures the transferase(s) which respond to induction by MC but not Pb (27). Both the rats consuming 10 ppm PBBs and their nursing pups were induced, by 120 and 240%, respectively. While this activity was also elevated in both mothers and pups in the 1.0 ppm group, the increases were not significant (Fig. 7).

A small number of pups from the 0, 0.1, and 1.0 ppm groups were maintained on their mothers' diets, raised, and allowed to mate. Their pups showed much the same responses to PBBs as did the original group of pups. The effects on both generations of adult female rats were also comparable (data not shown).

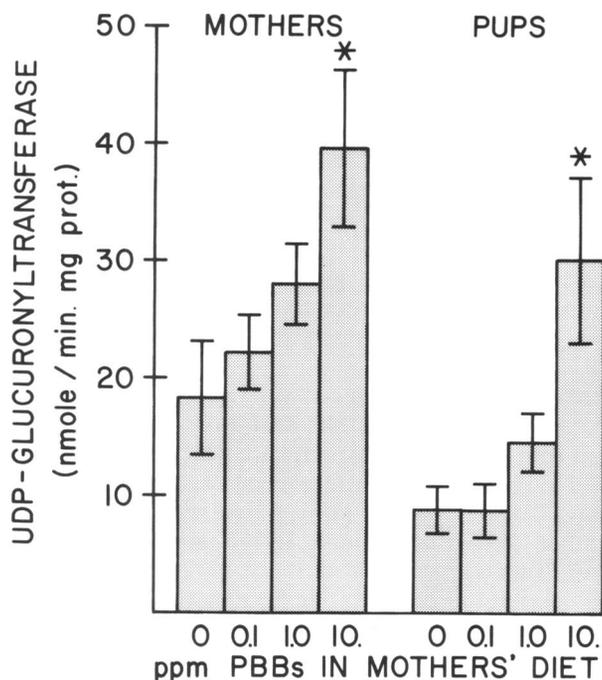


FIGURE 7. Effects of PBBs in the maternal diet on UDP-glucuronyltransferase in dams and pups. Details as in Figure 1.

## Discussion

Pb and MC are the two classic inducers of microsomal drug metabolizing enzymes. Pb increases liver weights, microsomal protein, NADPH-cytochrome P-450 reductase, cytochrome P-450, aminopyrine demethylation, and epoxide hydratase. It has little effect on UDP-glucuronyltransferase, when *p*-nitrophenol is used as the acceptor, or on benzo[a]pyrene hydroxylation. The

cytochrome P-450 spectral maximum remains at 450 nm. In contrast, MC has only small effects on liver weights and microsomal protein, and it has no effect on NADPH-cytochrome P-450 reductase, aminopyrine demethylation, or epoxide hydratase. It induces cytochrome P-450 hemoprotein(s) with spectral maxima at 448 nm, and is a strong inducer of both benzo[a]pyrene hydroxylation and UDP-glucuronyltransferase. These properties, as well as the distinctive electrophoretic profiles of microsomes induced by these agents, permit the effects of other chemicals on microsomal drug metabolizing enzymes to be characterized and classified. Conversely, the relative levels of these enzymes, as compared with their levels in control microsomes, can be used to establish the extent of exposure of animals to these two classes of inducers. Both approaches have been used in the experiments described in this paper.

On the basis of the pattern of effects caused by pure HBB<sub>6</sub> and HBB<sub>7</sub>, both major PBB components were classified as Pb-type inducers (3, 4). Similarly, PBBs were shown to be a mixed-type of inducer, because microsomes induced by PBBs displayed the properties of microsomes induced by both Pb and MC (1-7). One or more components in the remaining 17% by weight of FireMaster must be responsible for the MC-like aspects of the mixed-type induction caused by the PBB mixture. Poland and Glover have recently shown that 3,3',4,4',5,5'-hexabromobiphenyl is an excellent MC-type inducer (28); however, they did not address the question of whether this congener is found in PBBs. Whether it can, if present, account for the MC-like aspects displayed by PBBs is unknown.

The studies on lactating and nursing rats were designed, in part, to determine whether the full range of PBBs' effects on drug metabolizing enzymes could be transmitted through milk. At the time this work was initiated, it was known only that the compounds later identified as the Pb-type inducers HBB<sub>6</sub> (3) and HBB<sub>7</sub> (4) were secreted in cows' milk (17). The presence or absence of the other components from milk was not well defined (18). The pattern of enzymatic activities in nursing pups was therefore used as an assay for the presence or absence of Pb- and MC-type inducers in the milk.

As the results presented in this paper show, the ability of PBBs to cause a mixed-type induction of microsomal enzymes can be transmitted through rats' milk. Alvares and Kappas have reached the same conclusion for Aroclor 1254, a mixture of polychlorinated biphenyls (29). The increases in cytochrome P-450 and aminopyrine demethylation demonstrate that Pb-type inducers are transmitted

through the milk. HBB<sub>6</sub> and HBB<sub>7</sub> undoubtedly play a large role in causing this effect, since both are found in rats' milk in substantial quantities (10). It is also probable that a number of the other PBB components are Pb-type inducers as well, and that these contribute significantly to this aspect of the induction. The increase in UDP-glucuronyltransferase and the sharp increase in benzo[a]pyrene hydroxylation found in the pups also demonstrates that MC-type induction has occurred in the neonates, and that one or more unknown components responsible for these effects are therefore also being secreted in milk. At the highest dose given, the dams showed increases in all four of these parameters, demonstrating a mixed-type induction by direct feeding of PBBs, as expected.

The major purpose of the lactation and nursing study was to determine the dose-response relationship between PBBs and microsomal enzyme induction in both mothers and pups. Exposure to PBBs was only during the lactation period, and pups had no access to the PBB-supplemented feed, so any exposure to PBBs had to be through milk. Lactating rats fed 10 ppm PBBs had increased cytochrome P-450, aminopyrine demethylation, benzo[a]pyrene hydroxylation, and UDP-glucuronyltransferase. The pups nursing from these mothers were significantly induced in these four parameters, and in addition displayed increased liver weights and microsomal protein. While dams fed 1.0 ppm PBBs showed no significant inductions, their pups had increases in microsomal protein, cytochrome P-450, and in the two cytochrome P-450-catalyzed drug metabolism activities. No significant increases were observed in either the rats fed 0.1 ppm PBBs or their pups.

Sleight and Sanger have published the microscopic and ultrastructural analyses of liver samples taken from the animals used in this study (30). They found a wide variety of lesion in pups nursing from dams fed 10 ppm PBBs, and mitochondrial lesions in these dams and in the pups nursed by the 1.0 ppm PBB-fed rats. In agreement with the microsomal observations, no effects were observed in either the 1.0 or 0.1 ppm mothers, or in the pups nursing from dams fed 0.1 ppm PBBs.

When this study was extended for an additional generation at the 0, 0.1, and 1.0 ppm PBB levels, the results were comparable to those presented for the initial 18-day exposure. It thus appears that continuous exposure to these levels of PBBs, through milk and then solid feed from birth through maturity, does not markedly enhance or alter the induction pattern when compared with exposure during only lactation and nursing.

A related study by Dent et al. (31) showed that

liver weights, benzo[a]pyrene hydroxylation, and epoxide hydratase were induced in rat pups nursing from mothers fed 50 ppm PBBs for the 14 days following parturition. Comparable results were obtained following prenatal exposure for the same length of time, indicating that transplacental induction by PBBs can also occur (31). They also showed that, while benzo[a]pyrene hydroxylation was induced in mammary gland by PBBs, epoxide hydratase levels were decreased, a combination of enzyme levels which may affect the toxicity of compounds secreted in milk (32).

The results presented here indicate that nursing rats are affected more by PBBs than are their lactating mothers. There are several possible explanations for this observation. We have shown that the secretion of PBBs in rats' milk markedly alters their gas chromatographic profile (10). This could result in the pups receiving a mixture of PBBs which was more potent than that which was consumed by the dams, as a consequence of both the metabolism of congeners and of differences in the biological distribution among congeners. It is also possible that neonatal rats are inherently more sensitive to both microsomal induction and liver lesions caused by PBBs than are lactating rats. A third possibility is that nonlactating female rats of the same age and weight as the dams would have been fully as sensitive to PBBs as were the pups, but because the dams were able to reduce their body burdens through lactation, they were functionally less sensitive.

Regardless of the reason for the apparently enhanced sensitivity of nursing rat pups to PBBs, it is clear that as little as 1.0 ppm PBBs in the diet of the adult rat suffices to induce drug metabolizing enzymes in the nursing pup. Because of the small number ( $N=4$ ) of animals tested at each dietary level, this experiment did not arrive at an unequivocal no-effect dose. Several statistically insignificant alterations were seen at 0.1 ppm PBBs which might possibly have been real given a much larger test sample. Even so, such changes would probably be relatively small, and it seems safe to conclude that the true no-effect dose for microsomal induction in nursing pups is somewhere in the neighborhood of 0.1 ppm PBBs in the diet of the lactating adult rat.

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