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Effects of PCBs and Related Compounds on Hepatocarcinogenesis in Rats and Mice

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Commercial mixtures of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) can cause hepatocellular carcinoma in rats and mice. Present evidence indicates that these chemicals act as promoters and not initiators of hepatocarcinogenesis. Our results show that Firemaster BP-6 (FM) and its nontoxic major congener, 2,2′,4,4′,5,5′-hexabromobiphenyl (HBB), act as promoters in the two-stage model for hepatocarcinogenesis devised by Pitot and associates. A toxic congener, 3,3′,4,4′,5,5′-HBB, also was assessed for tumor-promoting activity. This congener, though not in FM, is similar to TCDD, in that both cause 3-methylcholanthrene (MC)-type induction of hepatic microsomal enzymes and produce similar toxic responses. FM contains several congeners which are mixed-type inducers in that they induce MC-type and phenobarbital (PB)-type enzymes. The toxicity of FM is most likely associated with its congeners which are mixed-type inducers and not to relatively nontoxic congeners such as 2,2′,4,4′,5,5′-HBB which are strictly PB-type inducers. Congener 3,3′,4,4′,5,5′-HBB acted as a tumor promoter only at a dose that was hepatotoxic. A synergistic effect on tumor promoting ability was produced by combining a nontoxic and nonpromoting dose of 3,3′,4,4′,5,5′-HBB with a promoting dose of 2,2′,4,4′,5,5′-HBB. Our results suggest that synergism between toxic and nontoxic congeners in FM may explain why mixtures such as FM have greater promoting ability than individual congeners. Our results also indicate that with PBB, toxicity and carcinogenicity are not necessarily related.

One of the major concerns of people exposed to environmental chemicals such as polychlorinated biphenyls (PCBs) is whether or not these chemicals are carcinogenic. Although epidemiologic studies have not conclusively shown a greater cancer risk for people as a result of PCB exposure, there is conclusive evidence that PCBs can cause hepatic cancer in rodents. Prolonged dietary administration of PCB mixtures has induced hepatic tumors in rats (*1–4*) and mice (*5–7*). Hepatocellular carcinomas were described in each species and, in addition, what are classified as neoplastic nodules and foci of cellular alteration were described (*8*).

Evidence to date strongly indicates that the mixtures of PCBs act as promoters of hepatic carcinogenesis. When PCBs were given to rats after an initiator such as diethylnitrosamine there was convincing evidence that they had a tumor promoting effect in the liver (*9,10*). Tumor promotion was also evident when impurities such as dibenzofurans were removed from the PCB mixture (*10*). Earlier, Kimura et al. (*11*) reported a high incidence of hepatocellular carcinomas in the livers of rats fed a diet containing a tumor initiator, 3′-methyl-4-dimethylaminoazobenzene, for 2 months followed by dietary administration of Kanechlor 400, a commercial mixture of PCBs, for 6 months. Since administration of the initiator by itself or administration of Kanechlor before or concurrently with the initiator did not result in hepatic tumors, the authors interpreted the results as indicative of a tumor promoting effect by PCBs.

Recently, emphasis has shifted to counting and measuring preneoplastic enzyme-altered foci in the liver as an indicator of the tumor promoting ability of chemicals such as PCBs. There is considerable evidence that these foci are precursor lesions of hepatocellular carcinomas (*12–17*) and they most likely represent the first histologically verifiable step in the sequential development of hepatic neoplasia. By comparing the number of enzyme-altered foci resulting from administration of the initiator alone with the number and area of foci resulting from administration of the initiator and then the suspected promoter, one can obtain an assessment of the tumor promoting effects of chemicals such as PCBs. By using such procedures, Deml and Oesterle (*18*) found that Clophen A 50, a commercial mixture of PCBs, had greater promoting effects in livers from female rats when compared to the effects in males. They also reported that weanling rats were much more susceptible to the promoting effects of PCBs than adult rats (*19*).

When studying PCBs and related chemicals as to their carcinogenic effects, the timing of administration is important (*20*). These chemicals are potent inducers

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of hepatic microsomal enzymes and these enzymes can metabolize many carcinogens to less potent metabolites. Therefore, if PCBs are given to rodents before the administration of hepatocarcinogens such as 3'-methyl-4-dimethylaminoazobenzene or diethylnitrosamine, an inhibitory effect on hepatocarcinogenesis can occur (*21*). Further studies are needed on the inhibitory or enhancing effects of PCBs on carcinogens.

The PBBs, as an environmental pollutant, came to public attention as a result of accidental contamination of animal feed in Michigan in 1973 (*22*). As a result of this accident, most people in Michigan have PBBs in their body tissues, and considerable public concern exists as to long-term effects such as cancer. Since PCBs and PBBs are closely related chemicals, it is not surprising that biological responses to these chemicals are nearly identical. Oral administration of PBBs has caused hepatocellular carcinomas in rats (*23,24*) and mice (*24*).

At Michigan State University we have studied the pathologic effects of PBBs in rats with special emphasis on the effects of purified PBB congeners (*25–27*). We have found that 2,2',4,4',5,5'-hexabromobiphenyl, the major congener in Firemaster BP-6 (FM), the commercial mixture of PBBs, is relatively nontoxic. This congener is strictly a phenobarbital (PB)-type inducer of hepatic drug metabolizing enzymes (*28*). Other congeners in FM such as 2,3,4,4',5-pentabromobiphenyl, 2,2',3,4,4',5-hexabromobiphenyl(HBB), 2,3,4,4',5,5'-HBB and 2,3,3',4,4',5-HBB, not only induce enzymes induced by PB but also induce enzymes induced by 3-methylcholanthrene (MC). These congeners, like the mixture, are classified as mixed-type inducers and most likely account for most of the toxicity of the commercial product (*29,30*).

We have used the Pitot protocol (*31*) to assess the tumor-promoting activity of PBBs because it clearly separates the initiation stage from the promotion stage. Initiation consists of a 70% partial hepatectomy of 200-g female rats followed 24 hr later by a subcarcinogenic dose (10 mg/kg body weight) of diethylnitrosamine (DEN). The promotion phase of the protocol consists of dietary administration of the test chemical starting 30 days after initiation. We use PB at a dietary concentration of 500 mg/kg as a standard tumor promoter and as a basis for comparison for our test chemicals. Tumor-promoting ability is assessed by counting and measuring enzyme-altered hepatic foci exhibiting γ-glutamyl transpeptidase activity. By using this protocol, we have demonstrated that FM and 2,2',4,4', 5,5'-HBB can act as tumor promoters in experimental hepatocarcinogenesis in rats (*32*). These results were predicted by Trosko and associates since the PBB mixture and 2,2',4,4',5,5'-HBB both inhibit *in vitro* cellular communication at noncytotoxic doses, a property of known tumor promoters (*33–35*).

In our assays, we found that diets containing 10 or 100 mg/kg of either FM or 2,2',4,4',5,5'-HBB had hepatic tumor-promoting ability. However, FM caused significantly more enzyme-altered foci to develop than did the purified congener. These results indicate that other congeners in FM are very effective as promoters or possibly the combination of congeners with mixed- or PB-type of microsomal enzyme induction have a synergistic or additive effect on promotion.

We have also assessed the capacity of 3,3',4,4',5,5'-HBB to act as a tumor promoter (*36*). This congener, though apparently not in FM, is of importance because it is similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in that both cause MC-type induction of hepatic microsomal enzymes and produce characteristic toxic and histologic responses (*27,37*). In Trosko's *in vitro* assay, this congener did not inhibit metabolic cooperation at noncytotoxic doses and, therefore, was predicted not to be a tumor promoter (*35*). Our studies in rats have shown that nonhepatotoxic doses of this congener (0.01 and 0.1 mg/kg in the diet) are not tumor promoters even though these doses elicit MC-type enzyme induction. A hepatotoxic dose of 3,3',4,4',5,5'-HBB caused severe hepatocellular enlargement and vacuolation and focal areas of necrosis. At this dose, tumor promoting activity was seen; the mechanism of which is most likely associated with hepatic cell hyperplasia secondary to chronic hepatotoxicity. We concluded that two mechanisms of tumor promotion by PBBs can occur. With the nontoxic congeners such as 2,2',4,4',5,5'-HBB, the tumor promoting effect is a direct one, possibly associated with interference in normal cell-to-cell communication. Toxic congeners may exert their effects less directly by causing chronic hepatotoxicity. Chronic or recurrent necrosis of hepatic cells can result in regenerative stimuli and be a mechanism of tumor promotion (*38*). Hepatic cells initiated by DEN may also be resistant to the effects of cytotoxic chemicals such as 3,3',4,4',5,5'-HBB and have an increased responsiveness to endogenous or exogenous growth stimuli (*39,40*). It is our contention that it is important to distinguish between tumor promoters that can exert effects at noncytotoxic doses and those that can only promote as a secondary effect of hepatic necrosis.

Kimbrough (*41*) hypothesized that since PCBs and PBBs are lipophilic and highly persistent in the body that it should not be necessary to feed these chemicals for extended periods to assess toxic or carcinogenic effects. She reported that a single dose of 1 g/kg of body weight or smaller amounts over a short period of time resulted in a high incidence of hepatocellular carcinomas in rats (*23*). We have also found that PBB given without an initiator enhanced the development of a small number of enzyme-altered foci. One interpretation of these results is that PBBs are complete carcinogens and, therefore, can act as initiators and promoters. Although these chemicals have not been shown to be genotoxic or mutagenic (*42–44*), as would be expected if they were initiators, the possibility of initiating activity cannot be ruled out as yet. However, carcinogenic activity of a promoter such as PBBs apparently can occur as a result of promotion of "environmentally

initiated" cells (*45,46*). We conclude that the lack of evidence that PBBs are mutagenic or genotoxic, the positive correlation of inhibition of intercellular communication *in vitro* and the ability to enhance development of enzyme-altered foci in an initiation/promotion system support our contention that PBBs act strictly as promoters of hepatocarcinogenesis. Recently we have obtained further evidence that PBBs act as promoters by showing that a summational effect is not seen when PBBs are given prior to DEN. An enhancing effect on the number of enzyme-altered foci occurs when PBBs are given after DEN (*47*). According to Williams et al. (*48*), promoters do not have a summational or enhancing effect when given prior to an initiator.

Kimbrough's observation that a single dose of PBBs —and likely PCBs—results in hepatocellular carcinoma in rats and recent work by us indicating that a single dose of PBBs can effectively act as a tumor promoter in an initiation/promotion assay have important implications. Classic tumor promoters of hepatocarcinogenesis such as phenobarbital are most effective when given frequently over extended periods (*49*). For persistent lipophilic compounds such as PBBs and PCBs, continual exposure from external sources is not needed for tumor promotion to occur.

We have produced a synergistic effect on tumor promoting ability by PBBs by combining a nontoxic and nonpromoting dose of 3,3′,4,4′,5,5′-HBB (0.1 mg/kg of diet) with a promoting dose of the nontoxic congener 2,2′,4,4′,5,5′-HBB (10 mg/kg in diet) (47,50). These results help explain why FM, at certain concentrations, has greater tumor promoting ability than individual congeners in our initial studies (*32*). An inhibitory effect on tumor promotion was seen when a toxic and promoting dose of 3,3′,4,4′,5,5′-HBB (1 mg/kg) was combined with a promoting dose of 2,2′,4,4′,5,5′-HBB (100 mg/kg). The results as to inhibitory effects may pertain to the fact that there is not a dose related effect on tumor promotion at higher concentrations of chemicals such as PBBs. For example, we see as many enzyme-altered foci after a single dose of 30 mg of the mixture of PBBs as we do with a dose of 150 mg and see greater numbers of foci with either of these doses than when rats are given 300 mg at a single dose in an initiation/promotion assay (unpublished observations).

The similarity of chemical structure and similar biological responses to exposure of animals to PBBs and PCBs raises the possibility that additive or synergistic effects on toxicity or tumor promotion may occur if people or animals are exposed simultaneously to these chemicals. People are especially concerned about this possibility because their bodies may contain PCBs, PBBs and other related chemicals such as dioxins. Studies are now in progress in our laboratory in which we are using a purified PCB congener, 3,3′,4,4′,5,5′-HCB, and a purified PBB congener, 2,2′,4,4′,5,5′-HBB, in combination studies to assess additive or synergistic effects on toxicity or tumor promotion. We hypothesize that our results will be similar to those seen in the combination studies with PBB congeners. We did not see an additive or synergistic effect on tumor promoting ability when we combined 1 mg/kg of FM with 1 mg/kg of Aroclor 1254, a commercial mixture of PCBs. However, dietary concentrations of 1 mg/kg of Aroclor 1254 significantly increased the number of enzyme-altered foci after only 100 days of feeding in the initiation/promotion assay we are using (unpublished observations).

An obvious question when one uses the Pitot model for initiation/promotion and kills the rats before there is evidence of hepatocellular carcinoma is whether or not carcinomas would develop if the animals were allowed to live for an extended time after the dietary treatment. To test this possibility, we have given rats FM at 10 mg/kg in the diet for 140 days and then fed a basal diet without FM for an additional 275 days. Although the number of rats was small, all four rats in this experiment had developed hepatocellular carcinomas whereas none given only the basal diet after initiation had tumors (*47*).

Our results are significant because they clearly show that nontoxic components of FM such as 2,2′,4,4′,5,5′-HBB can act as promoters of hepatocarcinogenesis in the rat. Therefore, with PBBs, and most likely with PCBs, toxicity and carcinogenicity are not necessarily related. Our results also indicate that interactions between chemically similar compounds with different biological effects such as 2,2′,4,4′,5,5′-HBB and 3,3′,4,4′,5,5′-HBB can have a potentiating or inhibitory effect on hepatic tumor promoting ability. We also believe it is important to distinguish between nontoxic compounds that may have direct tumor promoting activity and compounds which promote hepatic tumors secondarily to chronic hepatotoxicity.

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