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A COMPARISON OF A COMMERCIAL POLYBROMINATED BIPHENYL MIXTURE, 2,4,5,2′,4′,5′-HEXABROMOBIPHENYL AND 2,3,6,7-TETRABROMONAPHTHALENE AS INDUCERS OF LIVER MICROSOMAL DRUG-METABOLIZING ENZYMES

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Abstract—The commercial polybrominated biphenyl (PBB) mixture, Firemaster BP-6, is a mixed inducer of hepatic drug-metabolizing enzymes in the rat. Its effects resemble those of a combination of the phenobarbital and 3-methylcholanthrene classes of inducers. 2,3,6,7-Tetrabromonaphthalene (TBN) was studied as a prototype of the brominated naphthalenes which are present as minor contaminants of Firemaster BP-6. TBN is an isostere of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent known inducer of the 3-methylcholanthrene class. TBN is a 3-methylcholanthrene-type inducer; however, it is 10^4 times less potent than TCDD and it is only slightly more potent than Firemaster. The relatively low potency of TBN does not appear to be due to metabolism, since comparable amounts of TBN and Firemaster were found in the liver at equivalent doses. In contrast to TBN, the predominant component, 2,4,5,2′,4′,5′-hexabromobiphenyl (HBB), is a pure phenobarbital-type inducer. The no effect level for the effects of a single dose of Firemaster or 2,4,5,2′,4′,5′-HBB on hepatic microsomal enzymes was 8 μmoles/kg (4.7 mg/kg of Firemaster). When Firemaster was given chronically 5 days a week for 15–30 days, changes in hepatic enzymes occurred with doses as low as 0.3 mg/kg/day. Using liver enzyme activities as an index of hepatic change, a 30-day recovery study showed that the liver does recover partially after exposure ceases. The degree of recovery correlates with a decrease in the concentration of PBBs in the liver as PBBs are redistributed from the liver to the fat. Porphyria was not observed during the chronic experiment, but gross hepatic porphyria developed in the Firemaster-treated rats during the recovery period.

A commercial mixture of polybrominated biphenyls (PBBs) was manufactured and sold for use as a fire retardant under the trade name Firemaster BP-6. In 1973, accidental contamination of animal feed by this PBB mixture resulted in serious contamination of livestock and subsequent exposure of farm families in the State of Michigan [1]. PBBs produce a number of toxic effects when fed chronically to experimental animals, including thymic atrophy, microscopic alterations in the liver, and immunological changes [2].

Firemaster also induces a wide spectrum of hepatic mixed function oxidases, including those induced by the phenobarbital class of inducers (cytochrome P-450-dependent enzymes) and those induced by the 3-methylcholanthrene (3-MC) class of inducers (cytochrome P-448-dependent) [3]. A similar mixed-type of induction has been found with the commercial mixtures of polychlorinated biphenyls (PCBs) [4]. However, individual PCB isomers belong to one of two classes of inducers, resembling either phenobarbital or 3-MC, or they may be inactive [5]. It is of considerable interest to identify the component(s) of Firemaster which are responsible for the 3-MC-type induction, since the relative toxicities of PCBs, halogenated dibenzo-*p*-dioxins and dibenzofurans have been correlated with their capacity to induce a cytochrome P-448-dependent enzyme, aryl hydrocarbon hydroxylase [6,7].

Extrapolating from studies with PCB isomers [5,8,9], we would predict that biphenyls which contain halogens in three or more of the meta- and para-positions, but no substitutions in the ortho positions, will be 3-MC-type inducers, and that these isomers (e.g. 3,4,3′,4′-; 3,4,5,3′,4′-; and 3,4,5,3′,4′,5′-) will be much more toxic than other isomers. The major component (56% of the mixture) of Firemaster is 2,4,5,2′,4′,5′-hexabromobiphenyl (HBB) [10,11]. A number of brominated biphenyls and other minor components were identified, but none of the potentially more toxic PBB isomers have been found.

Commercial PCB mixtures are contaminated with ppm levels of chlorinated dibenzofurans [12]. Certain of the halogenated dibenzofurans and dibenzo-*p*-dioxins are extremely potent inducers of cytochrome P-448 [6,13]. The most potent of these, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is 30,000 times more potent than 3-MC [14]. Therefore, even ppm concentrations of halogenated dibenzofurans could contribute 3-MC-type component to the inductive effects of PCB or PBB mixtures. However, neither brominated dibenzofurans nor dibenzo-*p*-dioxins have been found in Firemaster PB-6 at a detection limit of 0.05 ppm [10].

Firemaster does contain approximately 200 ppm of brominated naphthalenes (penta- and hexa-isomers) [10]. The inductive effects of the halogenated naphthal-

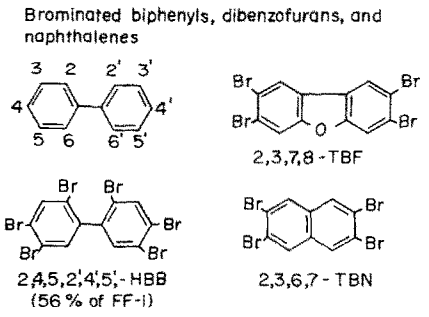

Fig. 1. Structures of brominated biphenyls and related compounds.

enes have not been studied; however, naphthalene halogenated in the 2,3,6,7-positions (Fig. 1) would be an approximate isostereomer of TCDD and might conceivably react with the same receptor. Morever, a number of penta- and hexachlorodibenzo-*p*-dioxins are known to be potent inducers of cytochrome P-448 [6]. The toxicology of the halogenated naphthalenes has not been studied systematically, but the early literature suggests that commercial mixtures of polychlorinated naphthalenes cause chloracne in man, x-disease in cattle, chick edema, and fatty liver and acute yellow atrophy of the liver in a number of species [15]. These symptoms are similar to those of TCDD poisoning [16]. Therefore, these compounds might conceivably be the components of Firemaster responsible for the 3-MC-type induction and much of the toxicity.

In the present study, we examined the effects of the commercial mixture, Firemaster BP-6, 2,4,5,2′,4′,5′-HBB, and 2,3,6,7-tetrabromonaphthalene (TBN) (Fig. 1) on hepatic microsomal enzymes to characterize the type of induction produced by each, to determine the relative potency, and to determine whether brominated naphthalenes account for the 3-MC component of the mixed-type induction seen with the commercial mixture. In addition, we examined the effects of Firemaster and 2,4,5,2′,4′,5′-HBB on hepatic microsomal enzymes during a 30-day exposure period, and at the end of a 30-day recovery period to determine a subchronic no effect level and evaluate the ability of the animal to recover from short-term exposure.

METHODS

Acute animal experiments. One-month-old female rats (Charles River, Fischer strain) (four per group) were injected i.p. with single doses of 1.8, 8, 40, 200 and 1000 μmoles/kg of Firemaster FF-1 or 2,4,5,2′,4′,5′-HBB in corn oil and decapitated 4 days later. The volume injected was 0.5 ml/100 g body weight. In one experiment, rats were injected with two daily doses of TBN (a total dose of 1.8, 6.25, 25, 100 and 400 μmoles/kg) in a volume of 2 ml/100 g body weight and killed 3 days after the second dose because of the relative insolubility of TBN in corn oil and its biological inactivity at low doses.

HBB and FF-1 were dissolved in corn oil with heat. TBN was dissolved in acetone, corn oil added, and the acetone removed under a stream of nitrogen. The average molecular weight of FF-1 was calculated from the per cent bromine (73.2%) determined by neutron activation. The value was corrected for 1.3% silica. These calculations yielded an average molecular weight of 577 (5.4 bromines/biphenyl) but must be considered approximate since they assume that the remaining 98.7% of the material is brominated biphenyl.

Chronic animal experiments. Chronic doses of 0.03, 0.3, 3 and 30 mg/kg of FF-1 or 0.16, 1.6 and 16 mg/kg of 2,4,5,2′,4′,5′-HBB were given p.o. in 0.2 ml of corn oil 5 days per week for 30 days to 7-to 8-week old female Fischer (F 344/N) rats obtained from the NCI-Fort Detrick breeding colony. Three rats from each treatment group were decapitated at each of four time points: 14 days (9 doses), 31 days (22 doses), 46 days (22 doses plus a 15-day recovery period) and 64 days (22 doses plus a 33-day recovery period). A few animals were killed at the 60-day recovery period (high dose only). The dose of HBB was chosen to represent the amount of this isomer in FF-1 at each of the three highest dose levels.

Chemicals. Firemaster FF-1 (lot no. FF-1312-FT) is a commercial mixture of brominated biphenyls obtained from the Michigan Chemical Co., St. Louis, MI, which consists of Firemaster BP-6 to which 1.3% silicate (Flo-gard) was added as an anticaking agent. FF-1 is the formula which was involved in the accident in Michigan. It was analyzed by gas-chromatography–mass spectrometry as described previously [10], and found to contain 56% 2,4,5,2′,4′,5′-hexabromobiphenyl in the fraction which eluted on the gas chromatograph. 2,4,5,2′,4′,5′-Hexabromobiphenyl (> 99 per cent pure) was purified from Firemaster BP-6 by repeated recrystallizations in either tetrahydrofuran-methanol mixtures or carbon tetrachloride. Sample purity was determined by gas chromatography (g.c.) on OV-101 (270°). The impurity was < 1%2,3,4,5,2′,4′,5′-heptabromobiphenyl.

A preliminary report on the method of synthesis of TBN was presented at the 175th National ACS meeting (March 1978) [17]. Full details will be presented separately. TBN was greater than 99 per cent pure, as determined by g.c. (OV-101) interfaced with a mass spectrometer. The only impurity (< 1 per cent) was a pentabromonaphthalene isomer. The methods employed for the synthesis of this compound preclude contamination by any oxygenated species (dibenzofurans, dibenzo-*p*-dioxins, phenols).

Analyses. Bromine analysis of tissues by neutron activation was performed after a 4-hr irradiation at 1.5×10^{13} n/cm^2-sec, with monitored decay, and a 400-sec count on an Ortec 24% Ge (li) detector coupled to a computerized ND 6603 MCA system. Standards consisted of six 1.2 μg bromine liquid solutions and six National Bureau of Standards standard reference materials containing approximately 1 μg bromine each. The approximate sensitivity to bromine was 1 μg bromine/sample and accuracy was ± 2 per cent. Hepatic aryl hydrocarbon hydroxylase (AHH) [14] and aminopyrine *N*-demethylase activities [18] were assayed in 9000 *g* supernatant fractions as described previously. Microsomes were prepared by $CaCl_2$ precipitation [19] and stored for 24–48 hr at −20° for determination of cytochrome P-450. Microsomal cytochrome P-450 [20] and ethyl isocyanide (EtNC) difference spectra [21] were determined in an Aminco DW-2

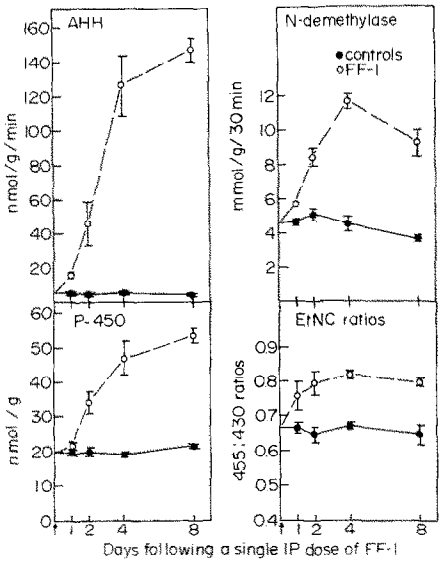

Fig. 2. Time course of induction of liver enzymes. One-month-old female rats were injected i.p. with a single dose of FF-1 and killed at the times indicated. Values represent means ± S.E. (N = 4).

spectrophotometer. The EtNC difference spectrum was obtained in 0.1 M potassium phosphate buffer at pH 7.4 by the addition of a saturating concentration of 1.1 mM EtNC to dithionine reduced microsomes. Tissue porphyrins were determined by the method of Abritti and DeMatteis [22].

Histology. Pieces of liver samples were fixed in buffered neutral 10% formalin for histophathologic examination, sectioned 6 μm thick, and stained with hematoxylin and eosin. Frozen sections of formalin fixed liver tissues were also stained with oil red O to identify and assess the accumulation of neutral lipid.

RESULTS

We used AHH activity, aminopyrine *N*-demethylase activity, cytochrome P-450 and alterations in the EtNC difference spectrum to differentiate between 3-MC- and phenobarbital-type inducers [23]. Phenobarbital-type inducers increase aminopyrine *N*-demethylase and cytochrome P-450 but do not alter the λ_{max} of the CO difference spectrum. Only minimal (4-fold) induction of AHH occurs with this class of inducers in our laboratory [5]. In contrast, 3-MC-type inducers increase AHH 20- to 50-fold, shift the λ_{max} of the CO difference spectrum to 448 nm, and increase the EtNC ratios, but decrease aminopyrine *N*-demethylase activity.

In a preliminary experiment, induction of AHH, aminopyrine *N*-demethylase and cytochrome P-450 were shown to be maximal 4 days after a single dose of 50 mg/kg of FF-1 (Fig. 2). In subsequent experiments, rats were killed at this time.

Figure 3 compares the dose–response curves for the effects of FF-1 and 2,4,5,2′,4′,5′-HBB. FF-1 is a mixed inducer. It increases both AHH and aminopyrine *N*-demethylase activities, shifts the peak of the CO difference spectrum and alters the ratio of the 455:430 nm peaks of the EtNC difference spectrum. Maximum

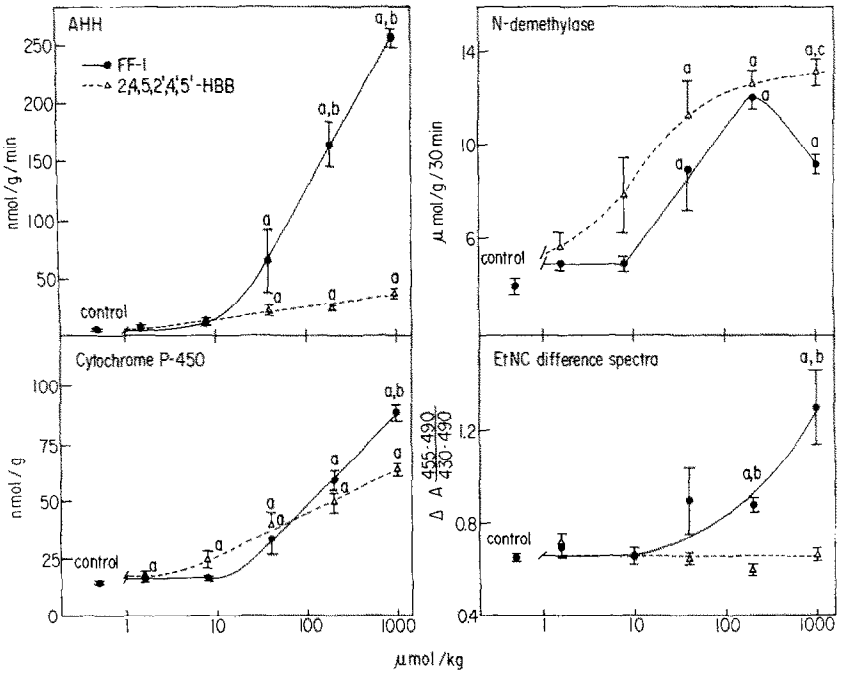

Fig. 3. Comparative dose–response curves for 2,4,5,2′,4′,5′-HBB and FF-1 on the induction of AHH, aminopyrine *N*-demethylase, cytochrome P-450, and the 455/430 spectra of reduced microsomes. One-month-old female rats were injected i.p. with single doses of HBB or FF-1 and killed 4 days later. Each point represents the mean ± S.E. (N = 4). Key: (a) significantly greater than controls ($P < 0.05$); (b) effect of FF-1 significantly greater than effect of corresponding dose of 2,4,5,2′,4′,5′-HBB ($P < 0.05$); and (c) effect of 2,4,5,2′,4′,5′-HBB significantly greater than effect of corresponding dose of FF-1.

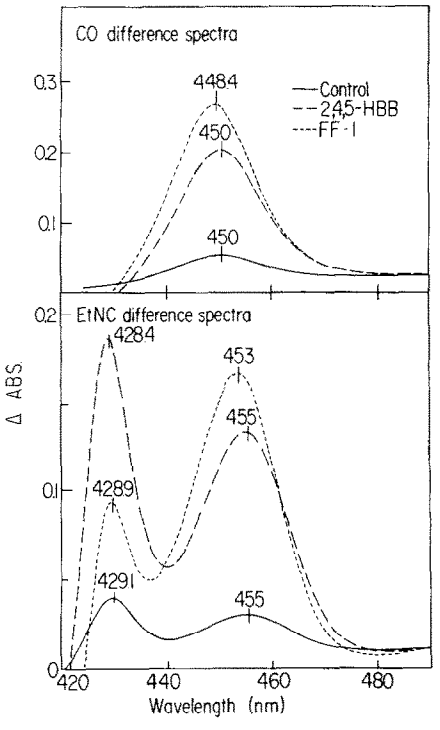

Fig. 4. Comparison of the carbon monoxide (top) and ethyl isocyanide difference spectra of reduced microsomes for control, FF-1- and 2,4,5,2′,4′,5′-HBB-treated rats. One-month-old female rats were killed 4 days after a single i.p. dose of 1 mmole/kg of FF-1 or 2,4,5,2′,4′,5′-HBB. Each difference spectrum represents a single rat. The EtNC difference spectra were determined in 0.1 M phosphate (pH 7.4) in the presence of 1.1 mM EtNC in a DW-2 spectrophotometer.

induction of AHH and the alteration in the spectral characteristics of cytochrome P-450 occur chiefly at higher doses. A dose of 1 mmole/kg (585 mg/kg) of FF-1 shifts the peak of the CO difference spectrum to 448.4 nm and alters the ratio of the EtNC peaks (Figs. 3 and 4). In contrast, aminopyrine *N*-demethylase is increased maximally at a lower dose (0.2 mmole/kg). A further increase in dose results in suppression of *N*-demethylase activity. 2,4,5,2′,4′,5′-HBB, on the other hand, resembles phenobarbital as an inducer. It increases aminopyrine *N*-demethylase, but produces only a slight increase in AHH and does not alter the spectral characteristics of cytochrome P-450.

Liver weight was increased 35 per cent by the highest dose of HBB; FF-1 produced a 78 per cent increase (Table 1). Neither compound affected body weight. Analysis of the livers of these animals for bromine indicates that equal amounts of PBBs were present in the liver of rats treated with 0.2 mmole/kg of FF-1 or 2,4,5,2′,4′,5′-HBB (Table 1). At the high dose, the amount of PBBs was higher in the FF-1-treated animals.

Figure 5 shows the dose–response curves for the effects of 2,3,6,7-TBN. TBN induced cytochrome P-448, as evidenced by a shift in the CO-difference spectrum to 448.5 nm, a 48-fold increase in AHH, a reversal of the EtNC ratios (Fig. 6), and a decrease in aminopyrine *N*-demethylase. TBN is approximately 10-fold more potent than FF-1; maximum effects were seen at 100 μmoles/kg (44.4 mg/kg). The approximate ED_{50} was 40 μmoles/kg (18 mg/kg). Equimolar doses of FF-1 and TBN produced comparable increases in AHH, but only FF-1 induced *N*-demethylase (Table 2). The concentration of bromine in the liver of the two

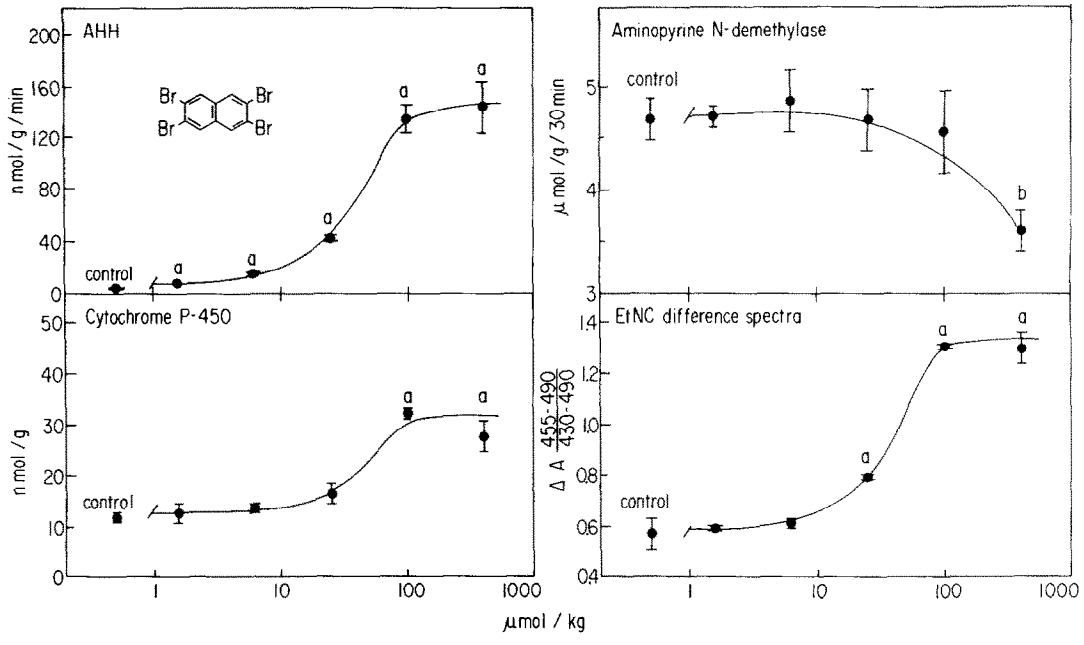

Fig. 5. Dose response for 2,3,6,7-TBN on AHH, aminopyrine *N*-demethylase, cytochrome P-450, and the 455/430 nm ratios of the ethyl isocyanide difference spectra at reduced microsomes. One-month-old female rats were injected i.p. with 2 daily doses of TBN (the total amount shown) and killed 3 days after the last dose. Each point represents the mean ± S.E. (N = 4). Key: (a) significantly greater than controls ($P < 0.05$); and (b) significantly less than controls ($P < 0.05$).

Table 1. Amount of bromine in FF-1- and HBB-treated livers

	Liver weight	Hepatic PBBs* (nmoles/g)
Controls	4.0 ± 0.2	
HBB (40 μmoles/kg)	4.2 ± 0.1	ND†
FF-1 (40 μmoles/kg)	4.4 ± 0.1	ND
HBB (200 μmoles/kg)	5.4 ± 0.2‡	89 ± 23
FF-1 (200 μmoles/kg)	4.9 ± 2.9‡	90 ± 31
HBB (1000 μmoles/kg)	5.2 ± 0.1‡	306 ± 51
FF-1 (1000 μmoles/kg)	7.1 ± 0.6‡	938 ± 241

* Total tissue bromine was determined by neutron activation. Calculated nmoles/g on the basis of the per cent bromine in the compound administered. The average bromine in the liver of the controls was (5.6 μg/g) subtracted from each group. Sensitivity was approximately 2 μg/g and accuracy ± 2 per cent.

† ND = not determined.

‡ Significantly greater than controls, $P < 0.05$, Student's t-test.

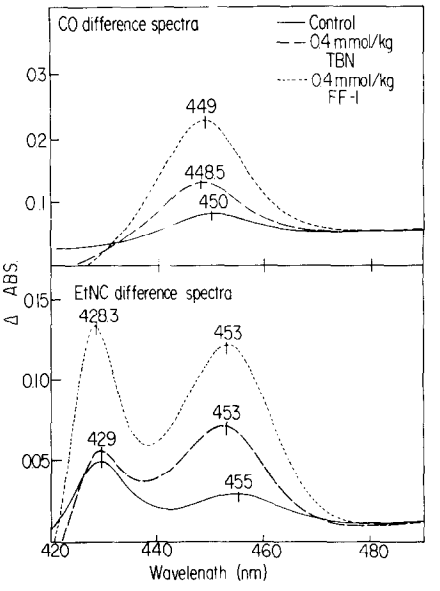

Fig. 6. Carbon monoxide (top) and ethyl isocyanide difference spectra of reduced microsomes from TBN- or FF-1-treated rats. One-month-old female rats were treated with 2 daily doses (i.p.) totaling 0.4 mmole/kg and killed 3 days after the last dose. Each difference spectrum represents a single rat. The EtNC difference spectra were determined in 0.1 M phosphate with 1.1 mM EtNC at pH 7.4.

groups was approximately equivalent. Body weight was not affected by this dose of TBN.

To check the possibility that the effects of brominated naphthalenes are potentiated by the presence of brominated biphenyls, we added TBN to 2,4,5,2′,4′,5′-HBB and compared the effects of the mixture with those of FF-1 and HBB alone (Table 3). The concentration of TBN chosen was approximately equal to the concentration of brominated naphthalenes in FF-1 (200 ppm). When 200 ppm TBN was added to 2,4,5,2′,4′,5′-HBB, the effects of the mixture resemble those of HBB alone, but differed from those of FF-1.

Liver histopathology. In rats given single doses of 0.4 or 1.0 mmole/kg of Firemaster FF-1, we observed moderate swelling of hepatocytes with hydropic degeneration in the liver, as denoted by vacuoles with rough

Table 2. Comparison of FF-1 and TBN*

Treatment	Aminopyrine N-demethylase	AHH (nmoles/g/30 min)	Cytochrome P-450 (nmoles/g/min)	EtNC 455:430 ratio	Liver wt (g)	Liver concn PBB or TBN† (nmoles/g)
Control	4.7 ± 0.2	3.1 ± 0.2	0.95 ± 0.07	0.57 ± 0.06	3.4 ± 0.01	
FF-1	13.6 ± 1.4‡	139 ± 20‡	3.59 ± 0.29‡	0.81 ± 0.03‡	4.5 ± 0.3‡	133 ± 56
TBN	3.6 ± 0.2‡	143 ± 20‡	2.24 ± 0.19	1.29 ± 0.06‡	4.6 ± 0.3‡	85 ± 24

* Rats were injected with 2 daily doses of 0.2 mmole/kg of FF-1 or TBN in 2 ml of corn oil/100 g body wt, and killed 3 days after the last dose. Values represent means ± S.E.

† Determined by neutron activation.

‡ Significantly different from controls, $P < 0.05$, Student's t-test.

Table 3. Evaluation of the possible additive effects of PBBs and TBN*

Treatment	Dose	Aminopyrine N-demethylase (nmoles/g/30 min)	AHH (nmoles/g/min)	Cytochrome P-450 (nmoles/mg protein)	EtNC 455:430 ratio
Control		2.7 ± 0.4	3.5 ± 0.6	0.83 ± 0.14	0.51 ± 0.01
FF-1	1 mmole/kg	7.2 ± 0.5†	149 ± 2	2.83 ± 0.19†	0.71 ± 0.08†
HBB	1 mmole/kg	10.2 ± 1.2†	16 ± 3†	2.60 ± 0.26†	0.49 ± 0.02
HBB + TBN	1 mmole/kg + 0.26 μmole/kg	10.5 ± 1.4†	15 ± 3†	2.60 ± 0.10†	0.50 ± 0.04
TBN	0.26 μmole/kg	1.8 ± 0.5	4.7 ± 0.3	0.77 ± 0.06	0.47 ± 0.03
TBN	0.2 mmole/kg	2.2 ± 0.3	57.2 ± 11†	1.83 ± 0.23†	0.77 ± 0.07†

* Rats were injected with 1 dose (2 ml/100 g body weight) and killed 4 days later. Values represent means ± S.E.

† Significantly different from controls, $P < 0.05$, Student's t-test.

Table 4. Histological changes in the livers of rats 4 days after a single dose of Firemaster FF-1 2,4,5,2′,4′,5′-hexabromobiphenyl (HBB) and 2,3,6,7-tetrabromonaphthalene (TBN)*

Compound	Dose (mmoles/kg)	Hepatic changes
Firemaster FF-1	1.0	Moderate swelling of hepatocytes, marked fatty infiltration (+++) around central vein, lipid globules moderate to large, hydropic degeneration.
Firemaster FF-1	0.2	Slight swelling of hepatocytes
Firemaster FF-1	0.04	Apparently normal
HBB	1.0	Slight to moderate swelling of hepatocytes, slight increase in number of fine lipid globules around central vein.
HBB	0.2	Slight swelling of hepatocytes but some of them appeared apparently normal.
HBB	0.4	Apparently normal.
Combined HBB and TBN	1.0 0.00026	Slight to moderate swelling of hepatocytes, slight increase in number of fine lipid globules around central vein.
Firemaster FF-1	0.4	Slight to moderate swelling of hepatocytes, occasional hydropic degeneration, moderate fatty infiltration (++) with fine globules around central vein.
TBN	0.4	Slight swelling of hepatocytes, slight fatty increase in number of fine lipid globules (+), occasional hydropic degeneration.
TBN	0.1	Apparently normal

* Rats were identical to those in Figs. 3–5 and Tables 1–3.

internal edges with a few cytoplasmic strands protruding in the middle (Table 4). Some hepatocytes contained large vacuoles with smooth borders, denoting moderate to marked fatty infiltration. These cells, which were located primarily around the central veins, contained fine to large oil red O positive neutral lipid globules. Occasionally, hyalinization was observed in the cytoplasm of a few hepatocytes. In a few cases, there was a marked increase in mitotic activity. The chromatin material of dividing cells appeared clumped together or was undergoing karyolysis. There were no obvious alterations in the livers of rats at lower doses.

Hepatocytes of rats given 0.2 to 1.0 mmole/kg of HBB showed slight to moderate swelling and increased mitotic activity. Hydropic degeneration was not observed. At lower doses, livers appeared normal. Hepatocytes of rats given 0.4 mmole/kg of TBN showed slight swelling of the hepatocytes, slight fatty infiltration, and occasional hydropic degeneration. On oil red O stain, the neutral lipid droplets were fine to medium in size, and were located primarily around the central veins. When 1 mmole/kg of HBB was given in combination with 200 ppm of TBN, the hepatic changes were similar to HBB alone. The marked fatty infiltration seen with 1 mmole/kg of FF-1 was not reproduced by this combination.

Chronic studies. When 3 or 30 mg of FF-1 was administered chronically, aminopyrine *N*-demethylase activity and cytochrome P-450 reached a maximum at 15 days (Figs. 7 and 8). Aminopyrine *N*-demethylase activity was significantly lower at day 30 (22 doses) than at day 15 (9 doses) although tissue levels rose with continued administration (see Table 6). At an intermediate dose (3 mg/kg), AHH activity rose throughout the 30-day treatment period, but at the high dose, activity was maximum earlier (15 days) (Fig. 9). Induction of these two enzymes occurred with doses as low as 0.3 mg/kg and was maximum with 30 mg/kg (Figs. 7–9). HBB accounted for most of the phenobarbital-type induction seen with FF-1, but not the 3-MC-type induction. The induction of AHH, aminopyrine *N*-demethylase and cytochrome P-450 was significantly less at 30 days than at 15 days despite continuous administration. Reversal of the EtNC ratios occurred only with the high dose of FF-1, and the cytochrome remained in this form even 30 days after dosing ceased.

Recovery of AHH activity, *N*-demethylase activity, and cytochrome P-450 was incomplete 30 days after the last dose (Figs. 7–9). Recovery was least at the high

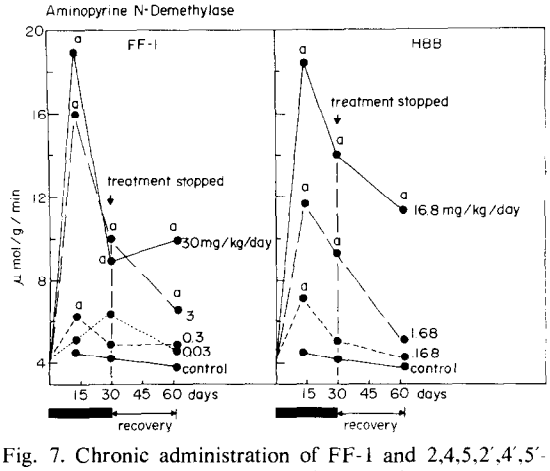

Fig. 7. Chronic administration of FF-1 and 2,4,5,2′,4′,5′-HBB on aminopyrine *N*-demethylase activity. Female rats were dosed orally 5 days/week for 30 days with designated doses of FF-1 or HBB. Groups of three rats per treatment group were killed at 14 and 31 days, and after 15 and 33 days of recovery. Values represent means. Key: (a) significantly different from control.

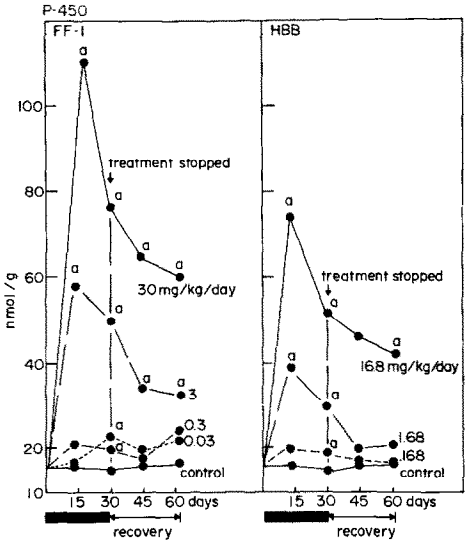

Fig. 8. Effects of chronic administration of FF-1 and 2,4,-5,2′,4′,5′-HBB on hepatic cytochrome P-450. Conditions are described in Fig. 7. Key: (a) significantly different from control.

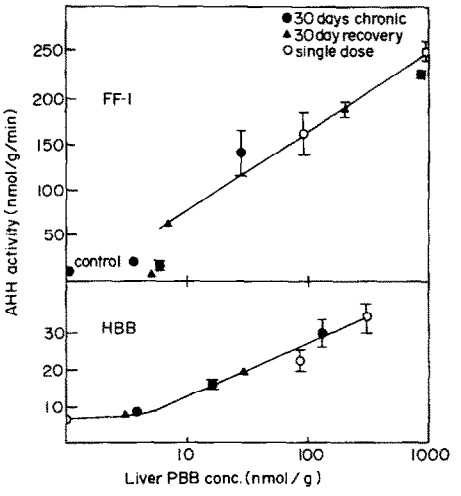

Fig. 10. AHH activity vs hepatic PBB concentration. The mean (± S.E.) AHH activity from each treatment group with FF-1 or HBB at 30 days, the 30-day recovery period (top 2 doses) or single dose (top 2 doses) is plotted versus the mean PBB concentration in the liver for each treatment group. AHH values are taken from Figs. 3 and 8, and PBB values from Tables 1 and 6.

dose (0–37 per cent for FF-1 and 16–47 per cent for HBB), but was generally greater at lower doses (50–80 per cent). Since dose–response curves generally yield a linear response when activity is plotted versus the log of the dose, we plotted mean AHH activity for each treatment group versus the log of the concentration of PBBs in the liver (Fig. 10). Values from the single dose studies, the chronic study, the 30-day dosing period, and the 30-day recovery period were plotted. A good correlation (> 0.99) was obtained for activity versus tissue concentration, indicating that the activity of AHH correlates with the tissue concentration of Firemaster during recovery.

Liver porphyrin concentrations in the female rats are shown in Table 5. Very little change in hepatic porphyrins occurred during the 30-day feeding period or the 30-day recovery period. After a 60-day recovery period, female rats dosed with the high dose of FF-1 were grossly porphyric. A similarly dosed group of male rats showed no alteration in hepatic porphyrins at identical time points.

The concentration of bromine in the liver and fat of rats dosed chronically with HBB or FF-1 is shown in Table 6. The concentration in fat did not decrease during the recovery period. In contrast, the concentration in the liver decreased 75 per cent during the 30-day recovery period, and an additional 75 per cent after 60 days. HBB and FF-1 appear to be distributed and stored in a comparable manner. The amount of PBB in the livers of the high dose FF-1 group was exceptionally high with respect to other groups.

Analysis of tissues for PBBs by neutron activation is compared with values obtained by g.c. analysis in Table 7. Gas chromatography values averaged higher (25 per cent) than neutron activation values. However, g.c.

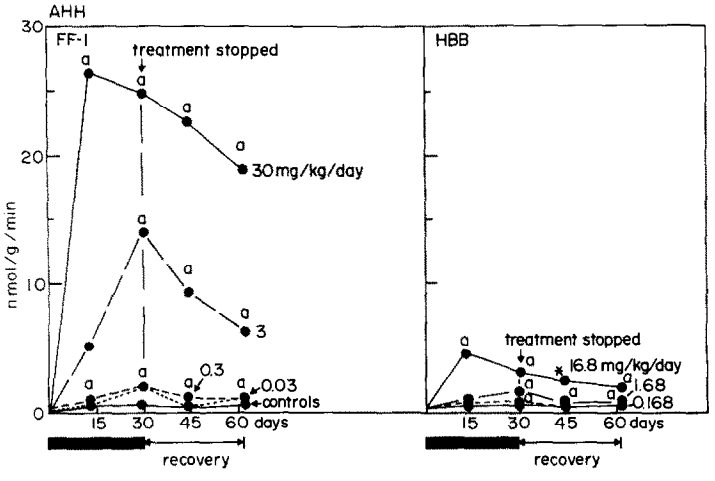

Fig. 9. Effects of chronic administration of FF-1 and 2,4,5,2′,4′,5′-HBB on AHH activity. Conditions are described in Fig. 7. Key: (a) significantly different from control.

Table 5. Liver porphyrins in female rats treated chronically with FF-1 or 2,4,5,2′,4′,5′-HBB*

Treatment (mg/kg/day)	Porphyrin (μg/g wet wt liver) 14-day treatment	31-day treatment	Porphyrin (μg/g wet wt liver) 15-day recovery	33-day recovery	64-day recovery
Controls	1.77 ± 0.11	1.57 ± 0.07	1.59 ± 0.06	1.53 ± 0.06	1.38 ± 0.05
HBB, 0.168	1.99 ± 0.09	1.87 ± 0.07†	1.64 ± 0.15	1.54 ± 0.07	
HBB, 1.68	2.40 ± 0.10†	2.17 ± 0.16†	1.79 ± 0.13	1.70 ± 0.08	
HBB, 16.8	3.07 ± 0.34†	2.01 ± 0.06†	2.40 ± 0.27†	1.56 ± 0.08	1.36 ± 0.03
FF-1, 0.03	1.95 ± 0.22	1.94 ± 0.10†	1.68 ± 0.15	1.52 ± 0.07	
FF-1, 0.3	1.89 ± 0.09	1.88 ± 0.09†	1.77 ± 0.19	1.47 ± 0.06	
FF-1, 3.0	3.11 ± 0.26†	2.64 ± 0.14†	2.19 ± 0.16†	1.78 ± 0.06†	
FF-1, 30.0	2.93 ± 0.16†	2.72 ± 0.06†	3.37 ± 0.13†	3.69 ± 0.28†	188 ± 75†

* Female rats were dosed orally 5 days/week for 30 days with FF-1 or HBB. Groups of three rats per treatment group were killed at 14 days, 31 days and after a recovery period of 15, 33 and 64 days. Values represent the means ± S.E. for three rats.

† Significantly different from controls, $P < 0.05$, Student's t-test.

Table 6. Bromine content of tissues from female rats dosed chronically with FF-1 or 2,4,5,2′,4′,5′-HBB*

Treatment (mg/kg)	Bromine content of fat (ug/g) 30-Day treatment	30-Day recovery	Bromine content of liver (ug/g) 15-Day treatment	30-Day treatment	30-Day recovery	64-Day recovery
HBB, 0.168	7.6 ± 0.2	14.4 ± 5.9	2.0 ± 0.6	2.4 ± 0.4	0.6 ± 1.0	
HBB, 1.68	42.0 ± 1.4	44.6 ± 1.3	4.0 ± 0.7	10.1 ± 1.3	1.9 ± 0.3	
HBB, 16.8	520 ± 61	334 ± 48	57.6 ± 4.6	81.6 ± 7.2	18.1 ± 1.0	8.9 ± 3.6
FF-1, 0.03	8.9 ± 3.5	22.8 ± 6.7	1.8 ± 1.3	3.4 ± 0.7	0.7 ± 1.0	
FF-1, 0.3	9.2 ± 1.7	6.7 ± 0.3	0.4 ± 0.2	2.1 ± 1.3	2.9 ± 1.5	
FF-1, 3.0	83 ± 6	62.2 ± 8.6	24 ± 14	16.2 ± 3.1	4.1 ± 2.0	
FF-1, 30	1014 ± 87	1075 ± 123	215 ± 14	500 ± 46	119 ± 22	34.3 ± 4.3
(Controls)	(2.3 ± 0.7)†	(2.4 ± 0.9)†	(5.6 ± 0.7)†	(3.9 ± 1.0)†	(5.1 ± 0.7)†	(4.9 ± 0.3)†

* Determined by neutron activation. Values represent means ± S.E.

† Control values were subtracted from treatment values.

Table 7. Comparison of neutron activation and gas chromatographic analysis of PBB in tissues of female rats dosed orally with 30 mg/kg/day of FF-1*

Tissue analyzed	Animal treatment	PBB (ppm) Neutron activation	Gas chromatograhpy	Hexa–Hepta-ratio†
Fat	30-Day treatment	1329	2500	4.6
		1596	1700	4.4
	33-Day recovery	1786	1700	5.3
		1410	1900	8.9
Liver	30-Day treatment	721	1000	3.2
		777	660	2.4
	33-Day recovery	228	310	9.4
		139	155	12.6
Control tissue + 6.5 ppm FF-1			6.8	1.3

* Values represent individual samples. Two portions of liver and fat were taken from each of four animals. One was analyzed by neutron activation and the other by gas chromatography. The treatment of the rats is described in Fig. 7.

† Ratio of 2,4,5,2′,4′,5′-HBB to 2,4,5,2′,3′,4′,5′-heptabromobiphenyl found in the gas chromatographic analysis of the tissue.

values are based on the major hexa-peak. The ratio of the hexa- to the hepta-peak is higher in tissue than in the standard, introducting some error into these estimates. This difference in peak ratios is even greater after a 30-day recovery period.

DISCUSSION

Firemaster BP-6 has been shown to be a mixed-type inducer, resembling a mixture of the two classical inducers, phenobarbital and 3-MC [3]. Since the completion of this work, Moore *et al.* [24, 25] have reported that the major components of Firemaster, 2,4,-5,2′,4′,5′-HBB and 2,3,4,5,2′,4′,5′-heptabromobiphenyl, which together comprise 83 per cent of this mixture, are phenobarbital-type inducers. Our work confirms their finding with the 2,4,5,2′.4′.5′-isomer, even at very high doses and after chronic exposure. These findings were not unexpected, since the corresponding PCB isomer is a phenobarbital-type inducer [5]. Relative rates of metabolism or distribution do not explain the differences between the effects of 2,4,-5,2′,4′,5′-HBB and Firemaster, since the concentration of PBB in the liver is comparable at equimolar doses. A disproportionately high concentration of PBBs was found in the livers of animals dosed with the high dose of Firemaster, but this was probably secondary to the accumulation of fat in these livers, since PBBs accumulate preferentially in fat. HBB does not produce a comparable increase in the amount of fat in the liver.

The present study is the first to examine the effects of the halogenated naphthalenes as inducers of the liver mixed-function oxidases. 2,3,6,7-Tetrabromonaphthalene (TBN) is an approximate isostere of TCDD; therefore we would expect that it might interact with the same receptor. Like TCDD, it is a rigid, planar molecule. The distance between the lateral halogens is considerably shorter for TBN (8.06 Å) than for TCDD (10 Å); however, bromine is more ionic than chlorine, and the additional electrostatic attraction could add to the potency of TBN. This seems to be the case in the guinea pig, where TBN has been shown recently to be 100 times less toxic than TCDD, but at least 100 times more toxic than the corresponding tetrachloronaphthalene [26].

As predicted, 2,3,6,7-TBN is a 3-MC-type inducer in the rat. However, it is 10^4 times less potent than TCDD and only slightly more potent than Firemaster itself. Although this isomer is not found in Firemaster, it should be the most potent of the brominated naphthalenes if structure–activity studies with chlorinated dibenzo-*p*-dioxins [6] are applicable. The penta- or hexabromonaphthalenes found in Firemaster could be more active than the 2,3,6,7-tetra-isomer if they are more resistant to metabolism. However, we found comparable amounts of TBN and Firemaster in the liver at equimolar doses. Therefore, it seems unlikely that rapid metabolism could explain the low potency of TBN. It should be noted that the tissue analyses were based on the amount of total bromine in the liver and could include metabolites. When 200 ppm of TBN was added to 2,4,5,2′,4′,5′-HBB, the effects were comparable to HBB alone. The HBB isomer did not potentiate the effects of this dose of TBN. Therefore, it seems unlikely that the 200 ppm of brominated naphthalenes present in Firemaster account for the 3-MC-type induction seen with Firemaster, although the penta- and the hexa-isomers found in Firemaster have not been tested. The relatively low potency of TBN with respect to TCDD is probably a reflection of lower affinity for the receptor because of the shorter distance between the lateral halogens.

Poland and Glover [8] have hypothesized that the TCDD receptor is a planar rectangle, 3 × 10 Å (a calculation which does not include the van der Waals radii of the halogens), and requires halogens in three of its four corners for finding. Biphenyls which are halogenated similarly but unsubstituted in the ortho-position (3,4,5,3′,4′,5′-hexa-; 3,4,3′,4′-tetra [5, 8]; and 3,-4,5,3′,4′-pentachlorobiphenyls [9]) apparently interact with the same receptor but are 10^3 to 10^4 times less potent than TCDD, presumably because there is an energy barrier to rotation [27] which must be overcome for the biphenyl rings to assume a coplanar configuration. This energy barrier is minimal for biphenyl, but will be increased considerably by the addition of even one ortho-substituent [27]. X-ray crystallographic measurements have demonstrated that some biphenyls can achieve coplanarity [28–31]. The only biphenyls which have been shown to achieve coplanarity either in their own crystalline lattice or when cocrystallized with selected compounds are those which have no ortho-substituents. A requirement for planarity would explain the findings that ortho-substituted biphenyls, e.g. 2,-4,3′,4′-tetra- [8], 2,3,4,2′,3′,4′-hexa- [5], and 2,3,4,-5,3′,4′,5′-heptachlorobiphenyls [5], are inactive as 3-MC-type inducers. Morever, when the biphenyl rings are fixed in a rigid planar structure, e.g. 2,3,6,7-tetrachlorobiphenylene, the potency is much greater than that of the corresponding biphenyl, and is, in fact, equivalent to that of TCDD [8].

PCB isomers which are 3-MC-type inducers and isotereomers of TCDD (e.g. 3,4,5,3′,4′,5′-hexachloro-) have been shown to produce a spectrum of toxicological changes similar to those of TCDD, while 2,4,-5,2′,4′,5′-hexachlorobiphenyl does not [6, 7, 32]. In the present study, liver histopathology also showed differences between the effects of single doses of Firemaster and HBB, indicating a difference in the hepatotoxicity of the two compounds. Only the Firemaster-treated animals showed hydropic degeneration and a substantial increase in fat. On the other hand, both compounds produce swelling of the hepatocytes consistent with increases in hepatic enzymes. It is difficult to assess the effects of TBN since limitations in solubility, the amount of compound, and the low toxicity prevented us from achieving a toxic dose, but hydropic degeneration was occasionally seen in the high dose animals. The pathological changes seen in the animals dosed chronically with Firemaster and HBB [2] are to be reported elsewhere. These changes (a decrease in body weight, atrophy of the thymus, and massive fatty infiltration of the liver) were not observed with 2,4,-5,2′,4′,5′-HBB.

In the chronic study, minimal changes in liver enzymes were seen with doses of Firemaster as low as 0.3 mg/kg/day, while maximum effects were seen with 30 mg/kg/day. Recovery of hepatic enzymes from the effects of PBBs correlated with the disappearance of the compounds from the liver. PBBs probably redistribute

from the liver into other tissues such as fat, since PBB levels in fat remain relatively constant during the recovery period. It is important that the liver did recover partially during the redistribution of these compounds into other tissues despite the fact that the amount in fat remained constant.

Chronic administration of PCBs, hexachlorobenzene and TCDD produces hepatic porphyria [33–35]. With each of these compounds, there is a lag period of 1–4 months before the porphyria develops. Several authors have hypothesized that the lag period reflects the time required for tissue levels to reach a critical level [36]. In this experiment, porphyria developed 60 days after the last dose of Firemaster, despite the fact that liver PBB levels decreased by 93 per cent during this period. The lag period and the massive amounts of porphyrin in the liver at 60 days indicate that PBBs produce a porphyria resembling the classical hexachlorobenzene-induced porphyria. A longer dosage period will be required to fully assess the dose-related porphyrogenic potential of FF-1.

It is important to identify the components of Firemaster responsible for the 3-MC-type induction, since presumably these compounds will also be the most toxic [6, 7, 32]. Our work indicates that brominated naphthalenes are 3-MC-type inducers. However, their potency is so low that the presence of 200 ppm of brominated naphthalenes in Firemaster is probably biologically insignificant, although the penta- and hexa-isomers have not been tested. 3,4,5,3′,4′,5′-HBB is a 3-MC-type inducer [8]. 3,4,3′,4′-Tetra-and 3,4,5,3′,4′-pentabromobiphenyls and brominated dibenzofurans would undoubtedly be active, since the corresponding chlorinated analogs are 3-MC-type inducers [5, 8, 13]. However, none of these compounds has been found in Firemaster [10, 11]. Several unsymmetrical PBB isomers (2,4,5,3′,4′,5′- and 2,4,5,3′,4′-) which contain one 3,4- or 3,4,5-ring, have been found in Firemaster [11]. Recent work by Dannan *et al.* [37] indicates that the 2,4,5,3′,4′,5′-hexabromo-isomer is a mixed inducer. However, this is difficult to reconcile with findings that similar unsymmetrical PCB isomers containing only one such ring (e.g. 2,3,4,5,3′,4′,5′-heptachlorobiphenyl [5] or 2,4,3′,4′-tetrachlorobiphenyls [8]) are not mixed inducers. Early evidence indicated that 2,4,5,2′,4′,5′-hexachlorobiphenyl was a mixed inducer, but the mixed induction was later shown to be the result of ppm levels of 2,3,7,8-tetrachlorodibenzofuran in the PCB isomer [38]. Alternatively, metabolism via debromination in the ortho-position might explain the results reported for the 2,4,5,3′,4′,5′-isomer.

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