vesicle opens 2000 channels, a single channel conductance is 20 pS, and the input resistance of the M cell is 200 kilohms favor the hypothesis that one rather than many vesicles is released by a single impulse at each synaptic bouton (H. Korn, D. S. Faber, A. Mallet, A. Triller, *Neurosci. Abstr.*, in press).

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# Hyperkeratosis Induced by Sunlight Degradation Products of the Major Polybrominated Biphenyl in Firemaster

Abstract. *Sunlight photodegradation of 2,2',4,4',5,5'-hexabromobiphenyl, the major component of Firemaster, gave a mixture that produces severe hyperkeratosis of the rabbit ear. This component in its pure state does not cause hyperkeratosis. One or more of the four major photolysis products must be responsible for this activity. A similar photodegradation pattern was observed for 2,2',3,4,4',5,5'-heptabromobiphenyl, the second largest component of Firemaster.*

In 1973 and 1974 more than 10,000 Michigan residents—principally farm families and their neighbors—were exposed to grain, meat, dairy, and poultry products that had been contaminated with a mixture containing polybrominated biphenyls (PBB's). The PBB's eventually were detected in a statistically representative sample of persons who had consumed this contaminated food (*1*).

In 1976 several government agencies funded a collaborative study between the Michigan Department of Public Health and the Center for Disease Control to assess and monitor the PBB exposure levels of various groups and adverse health effects. In addition, the Environmental Sciences Laboratory of Mount Sinai School of Medicine studied health effects among exposed farm populations and among workers at Michigan Chemical Company, formerly a producer of PBB's. These two groups showed a 3 and 13 percent incidence of chloracne, respectively (*2*). Chloracne, an acne-like eruption produced by prolonged exposure to certain halogenated aromatic compounds, may be accompanied by systemic toxicity (*2*). Compounds that cause chloracne also produce hyperkeratosis of the rabbit ear. The sensitivity of the rabbit ear to chloracne-causing compounds was first recognized by Adams *et al.* (*3*). The rabbit ear forms lesions exactly like those observed in human chloracne (*4*) and thus is an excellent model for studies of chemicals with this property (*2, 5*). Many investigators have used the rabbit bioassay to identify chemicals with chloracnegenic potential (*2, 5–10*), and we have used the test to assess hyperkeratotic activity in various chemicals (*11–14*).

In the collaborative study (*15*), the Michigan Department of Public Health and the Center for Disease Control have been monitoring the serum concentrations of 2,2',4,4',5,5'-hexabromobiphenyl (PBB-4), which constitutes approximately 60 percent of the commercial PBB mixture (Firemaster FF-1, lot FH 7042) involved in the Michigan PBB incident. However, this may not be the compound responsible for the toxic effects of Firemaster. We previously reported our attempt to identify the toxic components of Firemaster FF-1 by using purified congeners in the rabbit ear test (*12*). We report here studies on the sunlight degradation of certain PBB congeners, their keratotic effects, and PBB contamination of soil at the former manufacturing site in St. Louis, Michigan.

Table 1. Relative amounts of PBB-4 photolysis products at various intervals and rabbit ear test results. Total dose of reaction mixture per rabbit at each interval was 10 mg. Severe hyperkeratosis (+) consisted of dilation of the hair follicles, marked proliferation of the epithelium, and complete atrophy of the sebaceous glands. Abbrevations: N.R., no reaction; N.T., not tested.

| Exposure time (hours) | Amount of product in mixture (%) | | | | Rabbit ear test results |
|---|---|---|---|---|---|
| | PBB-1 | PBB-1a | PBB-2 | PBB-4 | |
| 0.0 | 0.5 | | | 96.0 | N.R. |
| 1.17 | 2.5 | 0.9 | 2.3 | 93.4 | N.T. |
| 2.34 | 4.2 | 1.1 | 4.4 | 86.0 | N.T. |
| 3.5 | 5.8 | 3.0 | 5.7 | 84.0 | (+) |
| 4.67 | 6.3 | 2.9 | 5.8 | 84.6 | N.T. |
| 5.84 | 7.0 | 3.7 | 5.7 | 76.4 | N.T. |
| 7.0 | 8.1 | 4.3 | 6.6 | 71.3 | N.T. |
| 8.17 | 9.7 | 5.1 | 8.7 | 70.8 | N.T. |
| 9.34 | 10.5 | 6.1 | 8.2 | 69.0 | (+) |
| 12.67 | 12.6 | 7.8 | 10.0 | 61.1 | N.T. |
| 17.7 | 14.7 | 8.7 | 9.2 | 52.5 | N.T. |
| 21.25 | 10.8 | 8.1 | 9.4 | 46.8 | (+) |
| 37.67 | 16.7 | 9.7 | 6.0 | 26.7 | (+) |
| 60.0 | 17.4 | 7.8 | 3.8 | 16.4 | (+) |

The photodegradation of PBB-4 by sunlight was conducted in a large quartz reaction vessel containing 0.3125 g of PBB-4 dissolved in 1500 ml of hexane. Portions were taken from the reaction vessel at various intervals for gas chromatography, gas chromatography–mass spectrometry, and rabbit ear tests. The results of the rabbit ear tests (Table 1) clearly show that although PBB-4 is not hyperkeratotic at the 10-mg dose, a severe reaction is produced if PBB-4 is exposed to sunlight for 3.5 hours. Portions removed from the vessel after 1.17 and 2.34 hours of sunlight were not tested for hyperkeratotic activity; therefore, 3.5 hours is not necessarily the minimum time required to produce severe hyperkeratosis.

Figure 1 shows the probable structures of the major photolysis products of PBB-4 (peaks 1, 1a, and 2) after 9.34 hours in the sunlight. The portion removed from the reaction vessel after 3.5 hours did not contain detectable amounts of peaks a, b, or c. Therefore, one or more of the PBB's represented by peaks 1, 1a, and 2 are probably responsible for the severe hyperkeratosis. Support for the structural assignments for peaks 1 and 2 can be found in earlier studies in which Firemaster was exposed to artificially generated ultraviolet light (*16–20*). In one of these studies (*20*), it was suggested that the major peak in the photolysis mixture (peak 1 in Fig. 1) corresponds to the loss of an *ortho* bromine, giving 2,3',4,4',5-pentabromobiphenyl (PBB-2). This PBB, however, was recently identified in Firemaster and characterized (*21*), and its relative retention time corresponds to peak 2 rather than peak 1. Peak 1 has the same retention time and mass spectrum as 2,2',4,5,5'-pentabromobiphenyl, which has also been identified in Firemaster (*21*).

Robertson *et al.* (*22*) showed that PBB-2 is a mixed phenobarbitone- and 3-methylcholanthrene–type inducer of hepatic microsomal enzymes. Loss of the *ortho* bromine by subsequent photolysis of PBB-2 would give peak 1a (tentatively identified as 3,3',4,4'-tetrabromobiphenyl), which has been reported to be a 3-methylcholanthrene–type inducer (*23*). Our gas chromatography–mass spectrometry data indicate that peak 1a represents a mixture of tetrabromobiphenyl and pentabromobiphenyl rather than pentabromobiphenyl alone, as reported earlier (*20*). Support for the tentative structural assignment of the tetrabromobiphenyl as the 3,3',4,4' congener can be found in the Cadagan coupling reaction between 3,4-dibromoaniline and 1,2-di-



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bromobenzene. The reaction mixture contains both of the possible products (2,3,3′,4′-tetrabromo- and 3,3′,4,4′-tetrabromobiphenyl), and the peak with the longer retention time has the same mass spectrum and retention time as the tetrabromobiphenyl in peak 1a (Fig. 1). The 2,3,3′,4′-tetrabromobiphenyl congener cannot be formed in the photolysis of PBB-4.

Photolysis of the chlorinated congener (2,2′,4,4′,5,5′-hexachlorobiphenyl) produced 3,3′,4,4′-tetrachloro- (70 percent) and 2,3′,4,4′5,5′-pentachlorobiphenyl (30 percent) (*24*). In a recently published capillary column chromatogram of Firemaster BP-6, there is a relatively large peak just after the peak corresponding to 2,2′,4,5,5′-pentabromobiphenyl (PBB-1) (*25*). This peak may represent the pentabromobiphenyl identified as 1a in the photolysis of PBB-4.

The major photolysis product of the second most abundant PBB in Firemaster (2,2′,3,4,4′,5,5′-heptabromobiphenyl, 22 percent) (*21*) is PBB-4, formed by loss of a *meta* bromine (*26*). In addition, a peak with the same retention time and mass spectrum as 2,3′,4,4′,5,5′-hexabromobiphenyl (PBB-6) is formed by the loss of an *ortho* bromine (*26*). This PBB-6 has also been shown to be a mixed inducer of hepatic microsomal enzymes (*21*). If this photolysis mixture is allowed to continue to react in sunlight, peaks are produced which correspond to 1, 1a, and 2 (Fig. 1). This photolysis mixture has not, however, been tested on rabbit ears.

In conjunction with these studies, we examined soil samples collected at the Saint Louis site by the Michigan Department of Public Health. One of the samples contained very high levels of PBB's (Table 2) for the peaks of interest. The relative amounts of PBB-1, -1a, -2, and -4 are different from those normally found in either Firemaster FF-1 or BP-6 and indicate apparent degradation of the Firemaster in this soil sample. The most dramatic difference is for peaks 1a and 2, where PBB-2 is actually larger than PBB-4. The identification of such a large amount of PBB-2 (a mixed inducer of hepatic microsomal enzymes) (*22*) in the soil sample is significant. The other two soil samples contained very low levels of PBB-4 (20 ppm) and the gas-chromatographic patterns were similar to those of Firemaster, indicating no significant degradation.

Table 2. Relative amounts of PBB congeners in commercial mixtures and in the highly contaminated soil sample. Values are percentages. Values in parentheses are parts per million.

| Sample | PBB-1 | PBB-1a | PBB-2 | PBB-4 |
|---|---|---|---|---|
| BP-6 | 0.07 | 0.0008 | 0.11 | 1.0 |
| FF-1 | 0.04 | 0.0005 | 0.02 | 1.0 |
| Soil | 0.09 (48) | 0.24 (128) | 1.1 (590) | 1.0 (530) |

This study has shown that compounds possessing hyperkeratotic activity can be produced from the two major compounds in Firemaster by sunlight degradation; these compounds constitute approximately 80 percent of the mixture. Except for using sunlight as an energy source, we did not conduct our study under field conditions. Nonetheless, our findings may have environmental relevance if PBB-contaminated soil exposed to sunlight comes in contact with a population. Such exposure is especially possible in areas around the former manufacturing site and around landfills containing large quantities of PBB's. The highly contaminated soil sample had considerably more of two of the PBB-4 photolysis products than the manufactured substance. These could have been formed by sunlight degradation. Our results indicate that in human health studies, PBB analyses should be based on selected toxic compounds rather than on PBB-4 (*15*). Since analogous reactions can occur in polychlorinated biphenyl mixtures (*24*), public health officials may wish to investigate the implications of these findings for the health of the populations exposed to surfaces contaminated with polyhalogenated biphenyls.

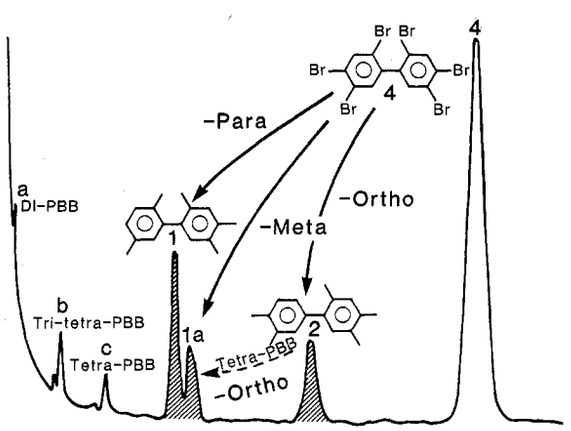


Fig. 1. Gas chromatogram of photodegraded PBB-4. The glass column (length, 6 feet; inside diameter, 1/8 inch; temperature, 220°C) was packed with 3 percent SP-2250 on Supelcoport. The gas chromatograph had a flame ionization detector.

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