diflubenzuron (in acetone), whereas in our work house fly egg hatchability per se was not seriously affected by 2 μg of topical diflubenzuron. Variation in the amount of unmetabolized diflubenzuron secreted into the eggs of the two house fly strains was probably the immediate cause of the observed differences in egg hatch. It may be that detoxication mechanisms were more active in the house flies used in our studies, thus leading to less availability for secretion into eggs.

Our studies strongly indicate that the ovicidal effects observed in adult insects exposed to diflubenzuron by surface contact or orally (Moore and Taft, 1975; Grosscurt, 1976; Taft and Hopkins, 1975; Wright and Harris, 1976; Wright and Spates, 1976) is due to secretion of unmetabolized diflubenzuron into the eggs, where it exerts its toxic effects upon the developing embryo or larva. The indication in our studies that treated male insects can transfer appreciable quantities of diflubenzuron to untreated females provides a logical explanation for previous reports of intersex transfer of "sterility" among topically diflubenzuron treated biting flies (Wright and Harris, 1976; Wright and Spates, 1976) and boll weevils (Moore and Taft, 1975). The transfer of diflubenzuron residues from topically treated male to untreated female flies is almost certainly due to surface contact between the sexes and not through seminal fluid. Grosscurt (1976) found that injection of male house flies with 5 μg of diflubenzuron on the day before mating with untreated females had no effects on egg hatchability.

Insecticide treatment of adult insects to effect secretion into the eggs and subsequent toxicity to the developing embryos has previously been reported as a potentially useful application of certain compounds (Masner et al., 1970). Diflubenzuron appears to have considerable promise for such use, and its relatively high degree of metabolic stability in insects seems desirable from the standpoint of minimizing the amounts required for effective control.

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Chemical and Toxicologic Evaluation of Firemaster BP-6

J. Ronald Hass,* Ernest E. McConnell, and Donald J. Harvan

The flame retardant (Firemaster BP-6) involved in an accidental contamination of animal feed in Michigan has been subjected to chemical and toxicological analyses to establish whether brominated dibenzo-*p*-dioxin or dibenzofuran impurities were present. Firemaster BP-6 was found to contain at least 13 different bromobiphenyls and to be contaminated with approximately 200 ppm bromonaphthalenes. A polar fraction, which should contain the majority of any possible bromodibenzofuran and bromodibenzo-*p*-dioxin contaminants, was prepared. Although the polar fraction contained a large number of components, no bromodibenzofurans or bromodibenzo-*p*-dioxins were found. In addition, the polar fraction was relatively inert toxicologically compared to the nonpolar fraction or the unfractionated material. It is concluded that the observed biological effects are most probably due to bromobiphenyls.

In 1973, the accidental contamination of animal feeds with a fire retardant (Firemaster BP-6) comprised of a mixture of polybrominated biphenyls (PBBs) resulted in serious animal toxicosis and loss of production (Jackson and Halbert, 1974). Secondary contamination became so widespread that by 1975 over 30 000 livestock, 1 600 000 poultry, and thousands of pounds of their products were destroyed to limit human exposure (Dunckel, 1975). The effects of polychlorinated biphenyls (PCBs) are complicated by the presence of low levels of the very toxic chlorodibenzofurans (Kuratsune et al., 1976a,b). Therefore, we examined the Firemaster BP-6 for the presence of similar contaminants. Moreover, O'Keefe

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(1976) reported the presence of bromonaphthalenes in a polar fraction of Firemaster BP-6, leading us to analyze for these compounds.

MATERIALS AND METHODS

The major constituents of Firemaster BP-6 (Michigan Chemical Co., St. Louis, Mich.) were analyzed by combined gas chromatography–mass spectrometry (GC–MS) using a Finnigan 9500 gas chromatograph/3300 mass spectrometer/6100 data system. The gas chromatograph was equipped with a 2 mm × 1.52 m glass column containing 3% OV-101 on 100/120 mesh chromosorb W-HP, programmed from 250 °C to 300 °C at 10 °C/min after an initial hold of approximately 40 s. Mass spectra were examined and the components partially identified on this basis.

Nuclear magnetic resonance (NMR) spectra were obtained using a Varian XL-100-12 spectrometer with Fourier transform and a 16 K 620/L computer using $CDCl_3$ as the solvent at room temperature. All chemical shifts are referenced to an internal standard of tetramethylsilane. Sample concentration of 100 mg/mL was used.

We analyzed unfractionated Firemaster BP-6 for brominated naphthalenes using selected ion monitoring GC–MS with the systems described above except the GC oven was programmed from 200 to 300 °C at 10 °C/min while monitoring m/e 444 (Br_4), 522 (Br_5), 600 (Br_6), and 680 (Br_7) using the MASS FRAG software. No GC peaks within proper retention time ranges were observed for m/e 444 or 680. Quantitative data were obtained by repeating the run while monitoring m/e 520, 522, 598, and 600 for penta- and hexabromonaphthalenes. Instrumental sensitivity was estimated through the use of bromonaphthalenes furnished by the NIEHS Organic Synthesis Program. The compounds were prepared by the treatment of naphthalene with Br_2 in the presence of $FeBr_3$ catalyst (Adams et al., 1963).

A polar fraction of Firemaster BP-6 was typically obtained by dissolving a 10-g sample in 150 mL of 3% methylene chloride (Burdick and Jackson; distilled in glass) in hexane (Burdick and Jackson) and chromatographing it on a Florisil PR Column (4 × 15 cm, 100 g of Florisil) which had been activated by heating at 130 °C overnight. The column was eluted with 500 mL of 3% methylene chloride–hexane to give a nonpolar fraction, then with 500 mL of 50% methylene chloride–hexane to give a polar fraction. The solvents were removed under vacuum in a rotary evaporator. The polar fraction was found to contain significant amounts of bromobiphenyls and was rechromatographed on a smaller column to give 17 mg of material (0.17% of original sample) which contained ≤0.1% hexabromobiphenyl.

An attempt was made to analyze the polar fraction by gas chromatography using a 2 mm × 1 m stainless steel column containing 3% OV-101 on 100/120 mesh Gas-Chrom Q. No peaks were detected within 45 min after injection of several micrograms of material at a column temperature of 300 °C. The sample was treated with Tri-Sil-Z (Pierce Chemical Co.) and analyzed gas chromatographically using a similar column 2 m long, again at 300 °C. As before, no peaks were observed for a period of 45 min.

Following the failure to analyze the polar fraction by gas chromatography, the material was reduced using 5% Pd on charcoal as the catalyst. Ethanol–tetrahydrofuran (THF) (1:1) and ethanol–methylene chloride (1:1) were evaluated as solvents. The latter combination gave a much improved solvent blank and polar fraction reduced in this solvent was used for further analysis. Typically, 10 mg of polar fraction was dissolved in 80 mL of solvent and hydrogenated overnight at 2.8 kg/cm^2 hydrogen pressure in a room-temperature hydrogenator. The reductate was filtered, the solvent removed under reduced pressure, and the final volume adjusted to approximately 1 mL with methylene chloride. A solvent blank was prepared by following the same procedure except that no polar fraction was used. The reductate was analyzed by combined GC–MS using a Finnigan 9500 gas chromatograph–3300 mass spectrometer system with instrument control, data acquisition and analysis performed by an INCOS 2300 data system. The gas chromatograph employed a 2 mm × 2.75 m glass column containing 3% SP2100 on 100/120 mesh Supelcoport (Supelco, Inc.) programmed from 100 to 280 °C at 10 °C/min after an initial hold of approximately 1 min. The injection port was maintained at 260 °C and the glass jet separator at 280 °C. The solvent blank was analyzed in the same manner. Each mass spectrum from the sample run was examined and key ion m/e's were selected. The data from the blank and sample runs were searched for the occurrence of these ions. Any mass spectrum from the sample run which occurred in the blank run with a similar retention time was not considered further.

The major hexabromobiphenyl was isolated by fractional recrystallization from THF–methanol. After three recrystallizations, a 2-g sample of Firemaster yielded 0.5 g of material which was 99.44% pure as determined by gas chromatography using a 3% OV-17 column (3.2 mm × 1 m).

High-pressure liquid chromatography was performed on Firemaster using a Waters Associates ALC-202 instrument equipped with M6000 pumps, solvent programmer, U6K injector, and a 25-cm μC-18 Bondapack column for analytical work or 9.5 mm × 0.6 m C-18 Porasil B column for preparative work. For analytical runs, a 95% methanol–5% water flow of 1.0 mL/min was maintained while 98% methanol–2% water at 5.0 mL/min was used for preparative work. The samples were dissolved in 20% tetrahydrofuran–methanol before injection.

After fractionation, an effort was made to determine if the polar fraction was toxic in vivo, using guinea pigs, mice, and rabbits. To accomplish this, two groups of six male Hartley strain guinea pigs weighing 250–300 g were given 1 mg/kg of the polar fraction in corn oil either by gavage or by subcutaneous (SC) injection. This dose of the "polar fraction" is equivalent to the amount of polar fraction in approximately 500 mg of the original Firemaster. Two groups of eight animals treated similarly with corn oil alone served as controls. In addition, a group of eight mice (8-week old male C57Bl/6) were given the corn oil polar fraction mixture by gavage so that each animal received 10 mg/kg of the polar fraction. Eight mice given corn oil only were used as controls. All of the above animals were housed individually and were provided food and water ad libitum. They were observed daily and were weighed three times each week for the duration of the study (30 days).

At the end of the study the animals were killed in a carbon dioxide chamber and complete necropsies were performed. Heart, liver, spleen, kidney, thymus, adrenal, and testicle weights were recorded. The livers were examined under ultraviolet light for the presence of red fluorescence as an indication of porphyrin accumulation. Portions of these and other organs were fixed in 10% neutral buffered formalin for later histopathologic examination.

Another study was undertaken using a standard ear painting procedure in rabbits similar to that described by

Table I. Polybrominated Biphenyls Found by GC-MS Analysis of Firemaster BP-6[a]

Compound	Percent area	Rel retention time
Pentabromobiphenyl	2.8	1.00
Pentabromobiphenyl	1.2	1.43
Hexabromobiphenyl	1.4	1.59
Hexabromobiphenyl	56.0	1.96
Hexabromobiphenyl	5.2	2.29
Heptabromobiphenyl	4.0	2.51
Heptabromobiphenyl	27.3	3.16
Heptabromobiphenyl	2.1	4.57

[a] The retention times are measured from time of injection. After an initial hold of 40 s the 3% OV-101 column was programmed from 250 to 300 °C at 10 °C/min.

Jones and Krizek (1962). In our study four groups of three adult (3 ± 0.5 kg) female New Zealand White rabbits each received five daily applications of either Firemaster, polar fraction, nonpolar fraction, or diluent (benzene–decane, 1:9). Firemaster and its fractions were dissolved at 40 μg/mL of diluent. One-tenth milliliter of each solution was evenly distributed over a 2 cm^2 area of the lower inside portion of the left ear. The opposite ear from each animal served as a nontreated control. The total dose of each compound at each application was approximately 1.3 μg/kg. After five daily doses the rabbits were observed for 10 days for the presence of epithelial lesions. At the end of this time they were killed in a CO_2 chamber, and both ears and the liver were fixed for subsequent histologic examination.

RESULTS AND DISCUSSION

Major Components. The total ion current plot from the GC–MS analysis of unfractionated Firemaster had eight discernible peaks. The mass spectrum corresponding to the maximum of each GC peak was examined. The empirical formula was readily deduced from the bromine isotope pattern and the molecular weight. The mass spectra were dominated by the molecular ions and ions corresponding to the successive loss of bromine. Table I lists the particular PBBs found, their fractional GC areas, and approximate retention times. If one assumes a relative response factor of unity for the various PBBs, one finds 4% penta-, 63% hexa-, and 33% heptabromobiphenyls which compares favorably with the percentage hexabromobiphenyl reported by O'Keefe (1976) from a direct probe analysis but different than the 90% hexabromobiphenyl (three isomers) and 10% pentabromobiphenyl (three isomers) reported by Norstrom et al. (1976).

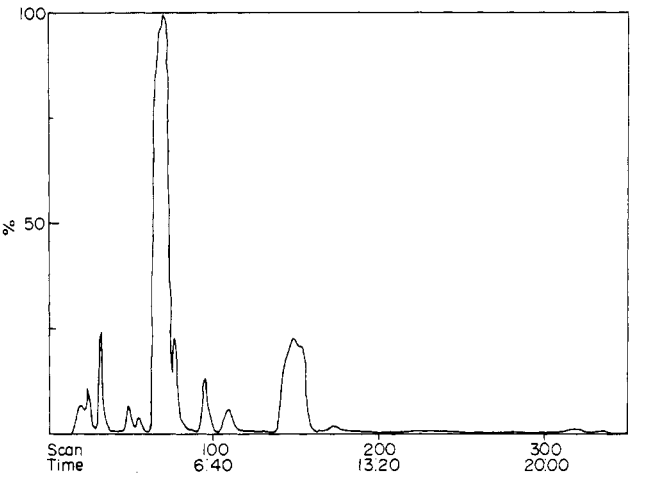


Figure 1. Total ion current reconstructed gas chromatogram for Firemaster BP-6 resulting from monitoring m/e 50–800 with 4-s cycle scans while programming a 3% OV-101 column from 250 to 300 °C at 10 °C/min.

Examination of the total ion current chromatogram (Figure 1) shows definite indications of column overloading. This severely limits the dynamic range of the analysis in that minor components may be lost in the tail of the major components. For this reason, the mother liquor remaining from the fractional recrystallization of Firemaster (and thus, depleted in major hexa- and heptabromobiphenyl) was itself fractionated using the reverse phase HPLC column and the various fractions analyzed by GC–MS. Chromatograms resulting from analytical and preparative scale injections are shown in Figure 2. Again mass spectra were examined. Components were considered identical if they had identical mass spectra and similar chromatographic retention properties. The unique compounds thus found total 13 PBBs (Table II).

The ^{13}C and ^{1}H NMR spectra of the major hexabromobiphenyl are shown in Figure 3. The ^{1}H spectrum consists of two singlets in the aromatic region (δ 7.40 and 7.88) which requires that the molecule contain two sets of two magnetically equivalent protons, i.e., that the bromine substitution pattern be the same for both rings. Further, the protons show a very small coupling constant which is indicative of a para arrangement. The ^{13}C spectrum confirms the symmetrical nature of the molecule. Using the method of Levy and Nelson (1972), one can estimate chemical shifts for substituted benzenes. Treating the possible symmetrical hexabromobiphenyls as substituted benzenes results in predicted values (Table III) for the

Table II. Polybrominated Biphenyls and Relative Peak Areas Resulting from GC-MS Analysis of the Preparative HPLC Fractionation of Mother Liquor Remaining from the Recrystallization of Firemaster BP-6[a]

No. of Br/HPLC fraction	1	2	3	4	5	6
5	19.9	6.1	0.7	0	2.5	4.7
5	2.1	2.7	0	0	1.6	3.1
6	10.6	0	0	0	0.8	1.4
6	24.0	57.1	68.5	18.2	23.5	33.4
6	32.3	16.8	2.3	3.6	6.5	12.7
6	0	0.7	1.8	31.9	36.0	14.4
7	6.3	5.3	3.6	0	0	5.3
7	1.7	0	0	0	0	0
7	0.4	10.1	21.3	46.3	17.1	21.3
7	2.7	0	0	0	0	0
6	0	0	1.7	0	0	0
7	0	0	0	0	6.3	3.7
8	0	0	0	0	5.8	0

[a] The HPLC fractions are as indicated in Figure 2.

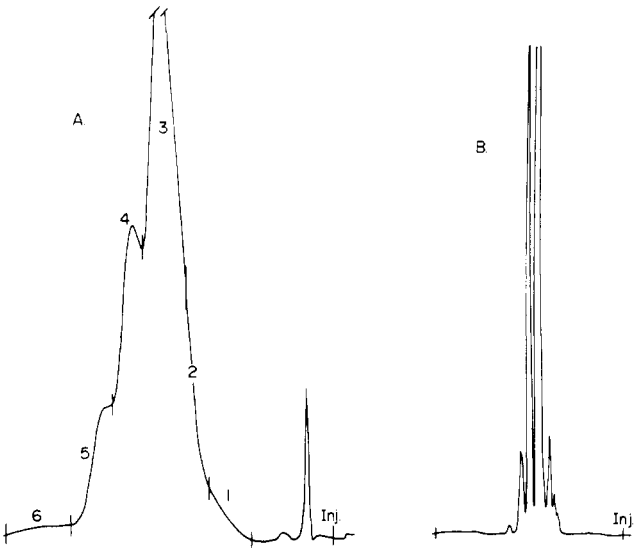


Figure 2. HPLC separation of Firemaster BP-6. (A) Preparative scale separation using 98% methanol–2% water through a C-18 Porasil B column. The numbers refer to the fractions collected for GC–MS analysis. (B) Analytical scale separation using the μC-18 Bondapack column.

protonated carbons. All except the 2,4,5 and 2,3,6 symmetrical hexabromobiphenyls can be eliminated on the basis of the ^{13}C NMR data. Since the 2,3,6 isomer has ortho protons, the correct structure is seen to be 2,4,-5,2′,4′,5′-hexabromobiphenyl, confirming the identification by other workers (Andersson et al., 1975; Sundstrom and Hutzinger, 1976). Comparison of the proton chemical shifts to those reported for the corresponding hexachlorobiphenyl (Wilson, 1975) leads to an assignment of 7.88 ppm to H-3,3′ and 7.40 ppm to H-6,6′.

Table III. Predicted Chemical Shifts for the Protonated Carbons for the Possible Symmetrical Hexabromobiphenyls Using the Method of Levy et al (1972)

Observed values, ppm	Calculated values			
	2,3,5,2′, 3′,5′	2,4,5,2′, 4′,5′	2,3,6,2′, 3′,6′	2,3,4,2′, 3′,4′
134.9	130.9	134.2	132.4	129.2
136.9	135.8	137.4	134.1	132.4

The ^1H NMR spectrum of the major heptabromobiphenyl consists of three singlets of equal intensity at 7.45, 7.47, and 7.93 ppm. The lack of splitting indicates that one ring has two protons with a 2,4,5 arrangement of the bromines as with the major hexabromobiphenyl. The chemical shift data, when compared to the assignments for the 2,4,5,2′,4′,5′-hexabromobiphenyl indicate the remaining proton is in the 6 position. Thus, the structure of the major heptabromobiphenyl is assumed to be 2,3,-4,5,2′,4′,5′-heptabromobiphenyl.

Polar Fraction. As noted in the Materials and Methods section, all attempts to analyze the polar fraction by GLC failed. Therefore, the material was analyzed by direct probe mass spectrometry. The resulting low resolution mass spectra were quite complex with ions observed the full range of the instrument. Although one could tentatively identify a few of the many components present, this approach was abandoned due, in part, to the likely misidentification of molecular ions.

The analysis of the polar fraction reductate by GC–MS resulted in a total ion current chromatogram with a number of peaks. The GC–MS data were searched for ions characteristic of dibenzofuran and dibenzo-*p*-dioxin. No evidence was found for either compound, indicating that the total concentration of bromodibenzofurans and bro-

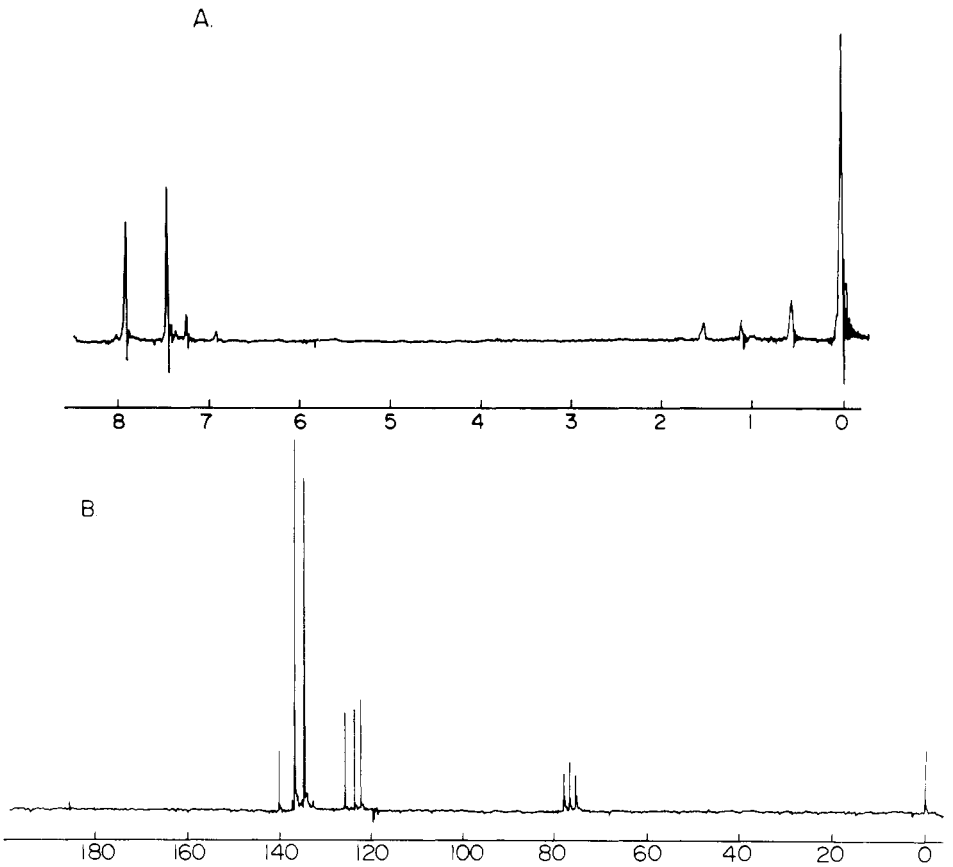
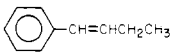
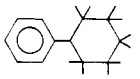
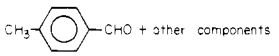
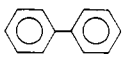
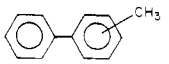
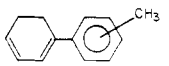
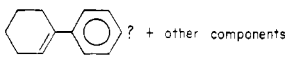
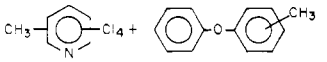
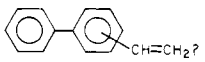
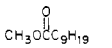
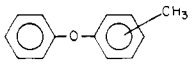
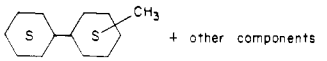
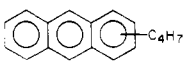
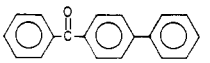


Figure 3. Proton (A) and ^{13}C (B) NMR spectra of the major component of Firemaster BP-6.

Table IV. Components of Firemaster Reductate Tentatively Identified on the Basis of GC-MS Data

Retention time, s	Tentative identification
384	
459	
519	Not identified
612	
633	
648	
702	Not identified
735	
756	
789	
798	$C_3H_5NCl_4$
849	
900	
915	
948	
975	
987	Fatty acid methyl ester
1050	Fatty acid methyl ester
1098	Fatty acid methyl ester
1104	Aliphatic hydrocarbon
1113	Fatty acid methyl ester
1224	
1257	

modibenzo-*p*-dioxins is less than 0.5 ppm. The majority of the compounds could not be positively identified from their low resolution mass spectra, but tentative identifications are shown in Table IV. Since the polar fraction was not particularly toxic (see below) when compared to either the nonpolar fraction or crude Firemaster, no further attempt was made to identify these compounds. Particular attention is called to the presence of the methyl biphenyls whose parent brominated compounds might be confused with bromodibenzofurans when present at levels near the mass spectrometric detection limits and the methyl diphenyl ether whose parent brominated compound might be similarly confused with a bromodibenzodioxin.

Bromonaphthalenes. Firemaster BP-6 was found to contain approximately 150 ppm pentabromonaphthalene and 70 ppm hexabromonaphthalene. These numbers must be viewed with caution since the particular bromonaphthalenes present in Firemaster were not available for instrument calibration. However, instrument sensitivity was established using bromonaphthalenes of the same bromine content. Since bromonaphthalenes have been reported in a polar fraction of Firemaster (O'Keefe, 1976), a mixture of these compounds was subjected to the same column chromatographic procedure used to fractionate Firemaster, both with pure bromonaphthalenes and as a 0.01% spike in Firemaster. In both cases approximately 98% of the spike material was recovered in the nonpolar fraction.

Biological Evaluation. The results for both the oral and SC exposure of guinea pigs were essentially the same. There was no significant difference between the controls and the animals exposed to the polar fraction with regard to clinical appearance, weight gain, organ weights (either absolute or relative to body weight), or on macroscopic or histologic examination of tissues. The same negative results were obtained in the mouse study.

The ears of rabbits treated with Firemaster BP-6, the polar fraction, the nonpolar fraction, or vehicle alone showed erythema within a few hours after the first administration of the compounds as a result of the irritating qualities of the benzene–decane solution. After 3 days, the treated areas of all the ears became scaly with exfoliation of small flecks of epithelium. By the end of the fifth day of exposure at least one rabbit in each of the four groups showed areas of yellowish hard plaques on a treated surface. At this time there was a slight accentuation of the hair follicles in one rabbit exposed to the nonpolar fraction. By the end of the study (10 days after the last exposure) all rabbits exposed to BP-6 and the nonpolar fraction showed mild but definite raised hair follicles in the treated areas. Other areas of the ear and the opposite ear remained normal. No follicle changes were observed in the ears of rabbits exposed to the polar fraction or the benzene–decane diluent.

Histologic examination of nontreated ears (all groups) showed the normal epithelium to be composed of two but more usually three cell layers. The ears from animals treated with the benzene–decane mixture and the polar fraction were similar to each other but thicker than nontreated areas; i.e., three to four cell layers. In contrast, four to six cell layers were observed in rabbits exposed to BP-6 and the nonpolar fraction. In addition, two of three animals exposed to the nonpolar fraction showed evidence of excess keratin and debris in the subjacent hair follicles. No evidence of chemical-related pathologic changes were found in the liver of any of the rabbits.

CONCLUSIONS

Firemaster BP-6 has been partially characterized chemically and a preliminary toxicological evaluation made with particular emphasis on the presence of extremely toxic bromodibenzofurans and bromodibenzo-*p*-dioxins. The dose levels of the polar fraction given to the guinea pigs and mice in this study were at least 10 times greater than the $LD_{50\text{-}30}$ of the more toxic dibenzo-*p*-dioxin isomers (McConnell et al., 1978) and dibenzofuran isomers (McConnell, 1977). The hyperplastic response of the nonpolar fraction and BP-6 in the rabbit ear test suggest that while these compounds have an acnegenic potential, the polar fraction does not. In addition, no chemical evidence could be found for the presence of dibenzo-*p*-dioxins and dibenzofurans in Firemaster BP-6 at a detectable limit of 0.5 ppm. Therefore, the above data indicate that these compounds are not present at significant levels in the polar fraction and that the toxic components of Firemaster are in the nonpolar fraction. The presence of bromonaphthalenes in the nonpolar fraction indicates a need for the evaluation of the toxic effects of these compounds. Since these compounds are present at concentrations not exceeding a few hundred parts per million at most, it seems likely that the bromobiphenyls them-

selves are responsible for the toxic effects observed. However, the importance of chronic low-level exposure to the bromonaphthalenes remains to be assessed.

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Toxicity of an Imported Fire Ant Bait Based on Phloxin B (D + C Red 27)

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Laboratory tests have shown that incorporation of Phloxin B (D + C Red 27) into a soybean oil–corn cob grit bait yielded an efficient toxicant for the imported fire ant. Phloxin B induces two lethal mechanisms within the imported fire ant: the first is light dependent and is effective in terms of hours; the second is light independent and is effective in terms of days. The relatively rapid light-dependent reaction is observed after a 1-day pretreatment which indicates that the reaction will not interfere with field collection of the bait by worker ants. Both toxic mechanisms were more pronounced in insects subjected to a slight energy stress caused by food deprivation.

The imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*, are major pests of a great portion of the southern United States. Current control measures are centered on the use of mirex as the active toxicant. Historically, the most used formulation has been the "4X-mirex bait" consisting of 850 parts corn cob grits, 147 parts soybean oil, and 3 parts mirex. This formulation has been cancelled, and other mirex-containing formulations are due to be cancelled effective December 31, 1977 (Federal Register, 1976). There is an urgent need for an improved method for controlling the imported fire ants.

Recently, certain dye molecules have been shown to be toxic to the imported fire ant (Broome et al., 1975a,b). The dyes were fed to the ants in the dark in an aqueous sucrose solution; and when the ants were subsequently exposed to visible light, mortality was observed in a matter of hours. If the ants were left with the dye in the dark for a longer period of time, never being exposed to light, mortality was observed in a matter of days.

In dye screening studies where inhibition of enzyme activity was the experimentally determined variable for dye-sensitized photooxidation, rose bengal was the most effective in every test (Callaham et al., 1975, 1977). However, when toxicity studies with the imported fire ant were performed, phloxin B (Figure 1) was very nearly as effective as rose bengal (Broome et al., 1975a). At first, this was attributed to experimental anomaly; but now, it is felt that phloxin B may simply be as toxic to fire ants as rose bengal for unknown reasons other than the efficiency of dye sensitization.

These above observations, coupled with the knowledge that some mammalian toxicological studies had been performed on phloxin B prior to its registration by the Food and Drug Administration as a drug and cosmetic (D + C) dye, led to the use of phloxin B as the basis for the new fire ant bait. Rose bengal is not currently registered as a D + C dye. It is felt that since some mammalian toxicological studies have been done with phloxin B, the registration of phloxin B as a pesticide may not take as long as for rose bengal. At the present time, two forms of phloxin B are registered with the Food and Drug Administration. The protonated form is D + C Red 27 and the disodium salt is D + C Red 28. D + C Red 27 was chosen for this application due to the fact that it is oil soluble while D + C Red 28 is not. The bait tested consists of corn cob grits, soybean oil, and phloxin B and is similar to the "4X-mirex bait". The results of both the light-dependent and the light-independent lethal reactions are presented here.

MATERIALS AND METHODS

Mounds of *S. richteri* were field collected and maintained in the mound soil in all-glass aquaria in the laboratory. A 5-cm strip of Fluon GP-1 (Northeast Chemical Co., Woonsocket, R.I.) was applied around the top of the inside walls of the aquaria for containment purposes. Water, but no food, was added daily to the mounds. After

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