Bull. Environm. Contam. Toxicol. 22,420-425 (1979)

Trace Contaminants in a Polybrominated Biphenyl Fire Retardant and a Search for These Compounds in Environmental Samples

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As a result of the accidental contamination of a cattle feed supplement in 1973 with a hexabromobiphenyl fire retardant, Firemaster FF-1, large numbers of cattle and other farm animals in Michigan became severely ill and many of the animals died (ROBERTSON and CHYNOWETH 1975, KAY 1977). Cattle mortality remained high in certain areas of the State, although polybrominated biphenyls (PBBs) in the animal tissues had decreased below the levels considered as significant from a human health standpoint by State and Federal regulatory authorities (CARTER 1976). The present study was undertaken with a view to determining if other brominated organic compounds of a more toxic nature than PBBs were present in Firemaster FF-1 or in environmental samples which may have been contaminated with Firemaster FF-1.

EXPERIMENTAL

Florisil chromatography was used as a first step for the isolation of polar compounds from Firemaster FF-1 (Michigan Chemical Co., St. Louis, Michigan). The procedure was a scaled down version of a similar step developed by VOS et al. (1970) and BOWES et al. (1973) for the separation of chlorinated dibenzofurans from polychlorinated biphenyls (PCBs). A 35 mg sample of Firemaster FF-1 was dissolved in 20 ml hexane and added to 30 g of suitably activated Florisil in a 15 mm i.d. column. The column was eluted with: (1) 100 ml hexane; (2) 75 ml 5% diethyl ether/hexane; and (3) 75 ml 25% diethyl ether/hexane. The third fraction was concentrated to 2 ml and then subjected to chromatography on a neutral alumina column (200 mm X 6 mm) followed by a microFlorisil column (40 mm X 6 mm). These two column separations were carried out by following the conditions described in a neutral procedure for the isolation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from bovine tissues (O'KEEFE et al. 1978). The complete neutral cleanup procedure of O'KEEFE et al. (1978) was used for the separation of polar brominated organic compounds from a trace mineral salt (38 g), feed scrappings from a dairy cattle barn (14 g), bovine eye fat (10 g) and bovine liver (40 g).

0007-4861/79/0022-0420 $01.20

Diazomethane was prepared by the method of ARNDT (1947). Reaction with Firemaster FF-1 was then accomplished by adding a 1 mg sample of the fire retardant in 1 ml hexane to 3 ml of an 0.5 M diazomethane solution in diethyl ether. After 1 hr at room temperature the solution was evaporated to dryness and taken up in a small volume of benzene in preparation for analysis by mass spectrometry.

All analyses were conducted with an AEI MS9 mass spectrometer. Samples were introduced into the mass spectrometer by capillary constricted glass tubes attached to a direct insertion probe (BAUGHMAN and MESELSON 1973 a, b). The mass spectral standard compound, perflurotributylamine (PCR, Inc.), was used for peak reference in both the low resolution and high resolution analyses. Spectra from dual ion monitoring in the high resolution mode were recorded after signal averaging or direct readout on a UV oscillographic recorder (BAUGHMAN and MESELSON 1973a, b). Low resolution spectra were recorded by the latter technique.

RESULTS AND DISCUSSION

(1) Firemaster FF-1

Low resolution (≈1,000) mass spectral analysis from m/e 200 to 850 showed that Firemaster FF-1 consisted of over 60% hexabromobiphenyl together with tetrabromobiphenyl, pentabromobiphenyl and heptabromobiphenyl. Bromobenzene compounds also appeared to be present although it was difficult to identify them positively because of the overlapping of their molecular ion clusters with those of the brominated biphenyl series. Two series of brominated compounds with signals appearing at 52 and 32 mass units lower than the corresponding brominated biphenyls were also recorded on the mass spectra. The identity of these compounds is not known at present. Some of the Firemaster solution was treated with diazomethane, a reagent which is known to methylate hydroxyl groups. There was no difference between the low resolution mass spectra from the diazomethane treated Firemaster and the untreated Firemaster. These results indicate that compounds such as phenoxy phenols and hydroxy biphenyls are not present to any significant extent in the Firemaster FF-1. Chlorinated derivatives of the former can serve as precursors of dibenzo-p-dioxins (RAPPE and NILSSON 1974) and halogenated derivatives of the latter could possibly give rise to the formation of dibenzofurans (NILSSON and RENBERG 1974).

In order to separate polar compounds from the major biphenyl components in the fire retardant, a 35 mg sample of Firemaster FF-1 was subjected to a multicolumn cleanup procedure. Although PBB compounds still predominated in low resolution mass spectra, two new brominated compounds containing 5 and 6 bromine atoms appeared in the spectra with molecular weights 26 mass units less than the corresponding PBBs. The molecular weights suggested that the compound were brominated naphthalenes and in fact chlorinated naphthalenes have been identified by VOS et al. (1970) and BOWES et al. (1973) in commercial preparations of PCBs. To provide additional evidence for the presence of naphthalenes, high resolution (9,000) peak matching was carried out for the principal isotopic ion (m/e 602) of the molecular ion cluster for hexabromonaphthalene. The spectrum, recorded on a signal averaging computer, had a peak at m/e 601.519 (Figure 1).

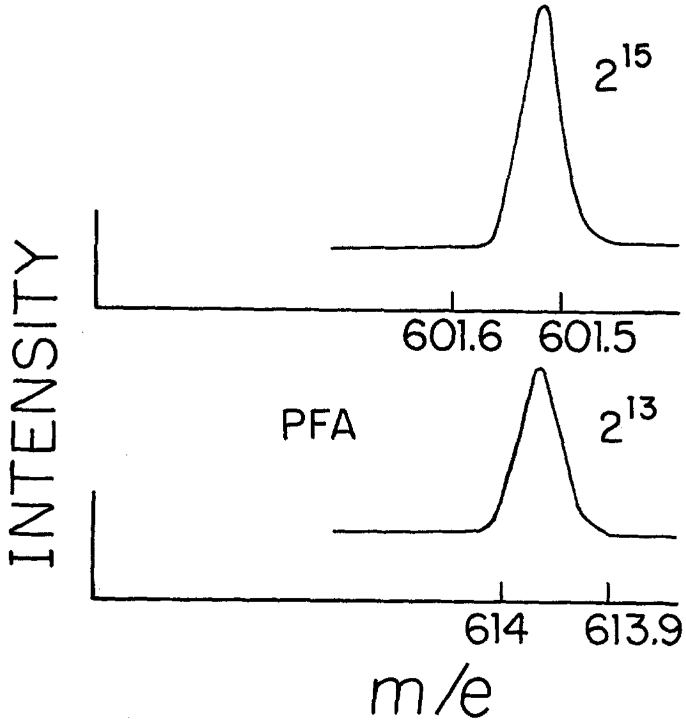

FIGURE 1. Analysis by dual ion direct probe high resolution (9,000) mass spectrometry of a cleaned up extract from Firemaster FF-1.

The theoretical value for hexabromonaphthalene ($C_{10}H_2Br_6$) is m/e 601.51945. When the peak matching controls were adjusted to monitor ions at m/e 602 and 604, the relative intensity of these ions (604/602) was found to be 0.76 (theoretical 0.73).

Since a standard sample of hexabromonaphthalene was unavailable. it was calculated that there was approximately 25 parts per million (ppm) of hexabromonaphthalene in Firemaster FF-1, by comparison to a standard sample of 2,3,7,8-tetrabromodibenzofuran (TBDF).

This compound volatilizes into the mass spectrometer source at the same temperature (290°C) as hexabromonaphthalene, and there is a strong probability that molecular ions are produced from the two compounds at approximately the same percentage of the total ion current. The material in the glass tubes was dissolved in benzene and cleaned up a second time on the alumina and microFlorisil columns. Over 60% of the hexabromonaphthalene was recovered and therefore it appeared that the cleanup method provided for a quantitative recovery of bromonaphthalenes. High resolution mass spectra were also recorded for the ions at m/e 521.611 and 523.609, the two major isotopic ions expected from a pentabromonaphthalene compound. The ion intensity ratio, 522/524 was found to be 1.06 (calculated 0.98). When TBDF was used as an external reference compound it was calculated that there was approximately 1 ppm of pentabromonaphthalene in the Firemaster FF-1.

Firemaster BP-6, an alternative formulation of Firemaster FF-1 without calcium silicate to prevent caking, has recently been analyzed by gas chromatography-mass spectrometry (HASS et al. 1978). Hexa- and pentabrominated naphthalenes were also identified in this study at levels of 70 ppm and 150 ppm respectively. These compounds were eluted in a nonpolar fraction from a Florisil column using a higher ratio of sample to adsorbent (100 mg/g vs 1 mg/g) and different eluents than in the present study. The differences in levels of brominated naphthalenes reported in each study may possibly result from obtaining samples from different production batches of PBB.

In a preliminary toxicological evaluation, HASS et al. (1978) found by means of rabbit ear skin tests that the naphthalene fraction and the unfractionated Firemaster BP-6 had some acnegenic potential. Since the materials were applied at equal concentration levels it was concluded that PBB compounds had probably produced the toxic effects. However it has been demonstrated that cattle inadvertantly exposed to chlorinated naphthalenes develop hyperperatosis and other symptoms (GREGORY et al. 1954) which are very similar to those prevalent in PBB exposed cattle (KAY 1976).

The high toxicity of certain chlorinated dibenzodioxins and dibenzofurans has been confirmed by many reports in the literature. Dibenzodioxins would not be expected to form readily in a mixture of biphenyl compounds, but chlorinated dibenzofurans have been detected in commercial PCBs (BOWES et al. 1975, VOS et al. 1970, NAGAYAMA et al. 1976). Single ion monitoring of the most abundant molecular ion isotopes was carried out by high resolution mass spectrometry for tetra-, penta-, and hexabromodibenzofurans in Firemaster FF-1. No detectable traces (>0.5 ppm) of these compounds could be identified under the conditions used in the present study. HASS et al. (1978) also did not find brominated dibenzofurans or brominated dibenzodioxins in Firemaster BP-6 at similar limits of detection.

(2) Bovine tissues and feed materials.

Samples of a mineral salt containing magnesium oxide, feed scrappings from a barn, and PBB contaminated[1] eye fat from two dairy cows and liver from one of the cows were subjected to a neutral cleanup procedure previously developed for the isolation of TCDD from bovine tissues (O'KEEFE et al. 1978). Low resolution mass spectral analysis failed to reveal any brominated organic compounds in the cleanedup extracts. In additional analyses of the liver and one eye fat sample (Cow #1), the m/e 602 ion of hexabromonaphthalene was monitored at high resolution but no signals were found in this mass region (Figure 2).

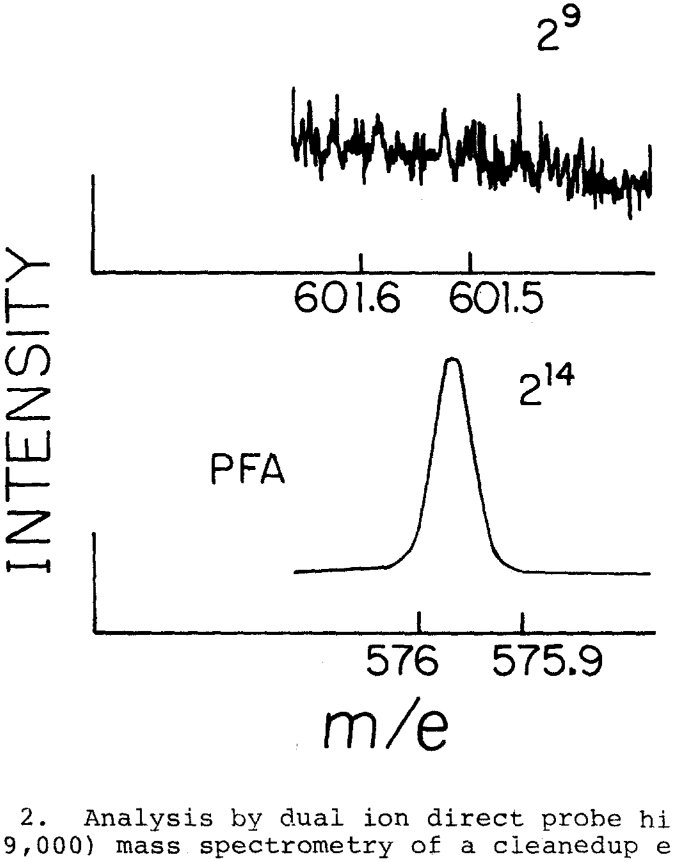

FIGURE 2. Analysis by dual ion direct probe high resolution (9,000) mass spectrometry of a cleanedup extract from bovine eye fat (Cow #1).

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[1] Levels of PBB compounds were determined by other laboratories using gas-liquid chromatography with the following results: eye fat cow #1, 0.08 ppm; eye fat cow #2, 0.46 to 1.69 ppm.

Although brominated naphthalenes were not detected in the environmental samples analyzed in this study, it is possible that these compounds could be detected with a mass spectrometer operating under more sensitive conditions. If in fact the possible contamination of environmental samples by brominated naphthalenes is based on the levels of brominated naphthalenes found in Firemaster FF-1, a detection limit of at least 2 parts per trillion would be required to determine their presence in the eye fat sample. However, at the time the mass spectrometer had a detection limit for TBDF of approximately 2 ng using dual ion monitoring under high resolution conditions. Since subsamples for analysis were generally equivalent to a 2 g fraction of the original sample prior to cleanup, this would lead to a detection limit of only 1 part per billion for brominated aromatic compounds in the environmental samples.

ACKNOWLEDGEMENTS

Funds for this work were provided by Mr. Gary Schenk, Grand Rapids, Michigan acting as attorney on behalf of a group of Michigan dairy farmers. Firemaster FF-1 and the environmental samples were obtained from Mr. Schenk and Dr. Alpha Clark, Mac Bain, Michigan.

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