The Finding of Chlorinated Dibenzofurans in a Japanese Polychlorinated Biphenyl Sample

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The presence of tetra- and pentachlorinated dibenzofurans and hexa- and heptachlorinated naphthalenes in two samples of polychlorinated biphenyl mixtures (PCB) of European origin has been reported by VOS et al. (1970a). Their interest in a content analysis of the two PCB samples for impurities was stimulated by previous work in which toxic effects observed in chicks were attributed to these two samples (VOS et al., 1970b).

Kanechlor 400 (KC-400), a commercial PCB preparation of Japanese origin, has been identified as the causative agent of Yusho, a human poisoning incident resulting from the ingestion of rice oil contaminated with KC-400 (KURATSUNE et al., 1972). We have analyzed a sample of KC-400 made available to us from the Kanegafuchi Chemical Industrial Co., Ltd. through Dr. H. Blumenthal of the Food and Drug Administration to determine whether potentially hazardous chemicals, particularly chlorinated dibenzofurans (Cl-DBF), were present as contaminants. It was the objective of this analysis to obtain, where possible, structural information on these contaminants and an estimate of their concentration in the KC-400.

Experimental

A hexane solution of the KC-400 was gas chromatographed, using an electron capture detector, on a column of 80-100 mesh Chromosorb WHP coated with 10% DC-200 and on a column of 15% QF-1 and 10% DC-200 each coated separately on 80-100 mesh Chromosorb WHP and mixed in a 1:1 weight ratio. The KC-400 (chlorine content 48%) showed only minor differences in peak locations and relative peak heights from Aroclor 1248 (chlorine content 48%), a PCB of American manufacture. Therefore, Aroclor 1248 to which a reference mixture of di-, tri- and tetrachlorinated dibenzofurans (prepared by chlorination of dibenzofuran in tetrachloroethylene with iron powder as catalyst) had been added, was used by Porter to devise a procedure to separate Cl-DBF from PCB (PORTER, 1971b). This procedure, as used in the present study, is outlined in Figure 1.

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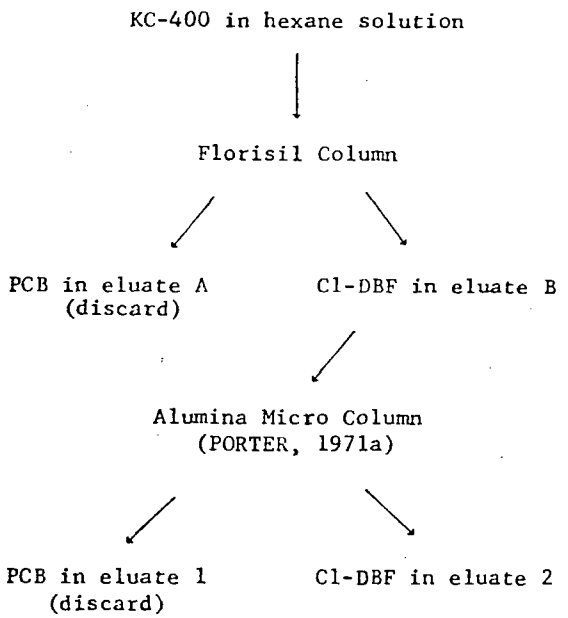

Figure 1. Procedure for Separation of Chlorinated Dibenzofurans from Chlorinated Biphenyls in KC-400

The Florisil (8 g of 60-100 mesh PR grade) was activated by heating at 130°C $\geq$ 12 hr and cooled to room temperature in a desiccator. Before the sample was added to the column, the Florisil column was prewashed with about 25 ml hexane. The KC-400/hexane solution ($<$ 2 ml) was placed on the Florisil, which was contained in a 10 mm i.d. x 300 mm glass column fitted with a Teflon stopcock and medium porosity fritted disk. The column was eluted with two eluants in succession at the rate of about 5 ml/min. Eluant A was 150 ml hexane. Eluant B was 100 ml 5% ethyl ether in hexane. Eluate B was concentrated to about 1 ml before the sample was placed on the alumina micro column.

The micro column was a disposable pipet of roughly 5 mm bore into which a small wad of glass wool had been inserted to retain the aluminum oxide. Merck© alumina, which had been heat-activated exactly like the Florisil, was poured into the column to a depth of 2.5 cm. Sample or eluting solvent was then added to the column from a disposable pipet or a large glass syringe. Eluant 1 was 10 ml 1% methylene chloride in hexane; eluant 2 was 6 ml 20% methylene chloride in hexane. Additional PCB was removed by concentrating eluate 2 to about 1 ml and subjecting it to a second

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alumina micro column cleanup, simply repeating the alumina column procedure.

This procedure was first applied to duplicate 2 mg portions of KC-400. Electron capture gas chromatography of eluate 2 showed a gas chromatographic peak in each sample that had a relative retention time matching that of a tetrachlorodibenzofuran on both a 10% OV-101 and a 1:1 10% DC-200 + 15% QF-1 column under the following conditions: glass column, 6 ft. x 4 mm id., column temperature 200°C, injection temperature 225°C; carrier gas, nitrogen at 120 ml/min.; detector, tritium source (concentric type), operated in electron capture mode at 210°C. Detector voltage was adjusted so that 1 ng heptachlor epoxide causes 1/2 full scale recorder deflection at 1×10^{-9} AFS.

The reference material for this retention time comparison was the tetrachlorodibenzofuran component in the mixture of chlorinated dibenzofurans.

To obtain sufficient material from the KC-400 for combined gas chromatography/mass spectrometry (GC/MS) analysis, the procedure was scaled up to permit cleanup of 8 mg portions of KC-400. Cleaned up eluates (eluate 2) were then combined to give two composite samples, each equivalent to approximately 100 mg KC-400. These two composites were analyzed by GC/MS (Finnigan 1015 quadrupole mass spectrometer) and the resulting data were examined for evidence of the presence of compounds that were not chlorobiphenyls. A reagent blank of equivalent size was also analyzed by GC/MS. The reagent blank contained none of the compounds found in the samples. The qualitative results of the KC-400 analyses are summarized in Table 1. The identification of the compounds listed in Table 1 was based on a comparison of the mass spectra of the sample compounds with the mass spectra of the di-, tri-, and tetrachlorinated dibenzofuran components in the reference mixture and on comparison with mass spectral data on chlorinated dibenzofurans in the published literature (VOS et al., 1970a; FIRESTONE et al., 1972; PLIMMER et al., 1973).

The amount of tetrachlorodibenzofuran contained in the reference mixture was approximated by comparing its microcoulometric GC response with the response given by a known quantity of a tetrachlorodibenzodioxin of known structure. The tetrachlorodibenzodioxin was considered an appropriate microcoulometric reference compound because of its structural similarity to tetrachlorodibenzofuran. The electron capture GC responses of the tetrachloro component in the synthetic mixture and in the KC-400 extracts were then compared to quantitate the tetrachlorodibenzofuran in the KC-400. The KC-400 was estimated to contain approximately 1 ppm tetrachlorodibenzofuran.

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TABLE 1

Compounds Present in Kanechlor 400 After Cleanup[a]

Mass (No. of Cl) and Fragmentation Data	Compound Present	Molecular Weight of Compound
256(3Cl);M-2Cl	trichlorobiphenyl	256
270(3Cl);M-(CO+Cl)	trichlorodibenzofuran	270
290(4Cl);M-2Cl	tetrachlorobiphenyl	290
298(5Cl);M-2Cl	pentachloronaphthalene	298
304(4Cl);M-(CO+Cl), M-(CO+3Cl)	tetrachlorodibenzofuran	304
312(5Cl);M-Cl	unidentified; perhaps methylpentachloro-naphthalene or pentachloro-phenyl cyclopentadiene	312
338(4 or 5Cl); M-(CO+3Cl) indicated	possible pentachloro-dibenzofuran	338

[a]Listed in order of elution from 3% OV-101, 6 ft. x 2 mm i.d. glass column at 220°C, carrier flow 20 ml He/min.; the same MS evidence was found in both samples examined for all entries but the last (possible pentachlorodibenzofuran) for which a second determination was not attempted.

Summary

Chlorinated dibenzofurans, including 1 ppm of tetrachlorinated dibenzofuran(s), and pentachloronaphthalene have been identified by combined gas chromatography/mass spectrometry in a polychlorinated biphenyl of Japanese manufacture (KC-400). The question of whether chlorinated dibenzofurans in KC-400 may have contributed to the reported Yusho incident is thus raised.

Acknowledgement

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