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Polychlorinated Quaterphenyls Identified in Rice Oil Associated with Japanese "Yusho" Poisoning

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Beginning in February 1968, about 1000 people in Fukuoka Prefecture, Japan, were poisoned as a result of ingestion of rice oil contaminated with polychlorinated biphenyls (PCBs). TSUKAMOTO et al. (1969) reported that rice oil responsible for the most severe clinical symptoms resulting from the "Yusho" poisonings contained 1020-1500 ppm chlorine, equivalent to 2125-3125 ppm PCB if calculated as Kanechlor 400, a Japanese commercial PCB mixture containing 48% chlorine.

The U.S. Food and Drug Administration (FDA)(1973) used these findings in assessing the health hazard associated with the ingestion of PCB in the establishment of tolerances for PCB in food and feed.

KURATSUNE et al. (1976) reported that subsequent measurement by gas chromatography (GLC) indicated that the rice oil actually contained 1000 ppm PCB, approximately 40% of the amount originally reported. In order to examine the rice oil more closely, FDA requested some of the sample from Dr. Kuratsune. Analysis in our laboratories showed this sample to contain 1000 ppm PCB, a value corresponding to the more recent Japanese figure.

Neutron activation analysis (NAA) of the sample, using the procedure for chlorine described elsewhere (TANNER and FRIEDMAN 1976), showed a level of 1050 ppm chlorine, twice as much as could be accounted for as PCB. A series of peaks other than those caused by PCB was seen when the oil sample (100 mg in 25 ml of isooctane) was gas chromatographed at conditions designed to speed the elution of chemicals through the column and permit examination of chemicals with high boiling points. The GLC conditions were: 3 foot x 2 mm i.d. column, packed with 1% OV-17 on Chromosorb WHP; temperature programmed from 230 to 270°C at 4°/min, with final temperature held for 30 min; constant current electron capture detector. At these conditions, decachlorobiphenyl (DCB) eluted in about 1.75 min and the polychlorinated terphenyls (PCTs) in Aroclor 4465[a] eluted as a partially resolved mass in about 6-12 min. The unidentified compounds in the rice oil eluted as an unresolved mass at about 7-19 min with the maximum response occurring at about 12 min. Comparison of the magnitude of response of the electron capture GLC detector to these unknowns and to Aroclor 4465 indicated that if

[a]Aroclor 4465 is a mixture of polychlorinated biphenyl and polychlorinated terphenyl; the mixture contains 65% chlorine.

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the unknowns were chlorinated aromatic compounds they could contain the 500 ppm chlorine present in the rice oil that was not in the PCB.

To facilitate identification of the unknowns, they were separated from the PCB residue by chromatography of a solution of the rice oil on a Florisil column (PAM 1971). Approximately 95% of PCBs and PCTs eluted from the column in the first fraction (fraction A; 250 ml of petroleum ether); the unknowns eluted in the second fraction (fraction B; 200 ml of 6+94 ethyl ether-petroleum ether).

The Florisil fractions from the "Yusho" rice oil sample were analyzed for chlorine by NAA. Fraction A, the PCB fraction, contained 400 ppm chlorine, roughly equivalent to the 1000 ppm PCB already calculated to be in the oil. Fraction B, containing the unknowns, contained 440 ppm chlorine and thus accounted for the amount of chlorine which was not associated with PCB in the oil.

To confirm the presence of the unknown contaminant, additional rice oil samples also associated with the Yusho incident were obtained from Drs. Akio Nakamura and Yoshito Masuda. GLC analysis of these samples showed them to contain 800 and 200 ppm PCB, respectively. The unidentified contaminant was found in both samples and was in essentially the same proportion to PCBs as in the original sample.

The unknown contaminant in fraction B was examined, using a Finnigan 3300 gas chromatograph/mass spectrometer (GC/MS) with data system. The MS was operated in the electron impact mode. The GC/MS conditions were: 2 foot x 2 mm i.d. column packed with 2% OV-101 on Chromosorb WHP; temperature 250° until 1 min after injection, then temperature programmed 1°/min, 250 to 272°C, then 4°/min to 300°C. Other temperatures were set as follows: separator, 280°C; transfer line, 272°C; analyzer, 50°C. Evaluation of the mass spectral data indicated that several of the unknown contaminants could be polychlorinated quaterphenyl compounds with 6 to 10 chlorine atoms. However, failure of the GLC system to separate the unknown contaminants adequately made further compound identification impossible.

Because the mass spectrometry of the unknowns was impeded by the lack of resolution of these substances by GLC, fraction B containing the unknowns was perchlorinated by the procedure of ARMOUR (1973) in an attempt to produce derivatives more readily separated. GLC analysis of the perchlorinated fraction produced a chromatogram with seven distinct peaks, all eluting in about 30-60 min. GC/MS analysis of this perchlorinated mixture indicated that two of the major components were perchlorinated quaterphenyl compounds and gave strong evidence that several other peaks were also perchlorinated quaterphenyls.

Crude grade quaterphenyl was obtained from the Monsanto Chemical Corporation. Analysis of this material by GC/MS showed that it was a mixture of quaterphenyl isomers. Following perchlorination, GLC retention times of the peaks from the perchlorinated crude grade quaterphenyl closely matched those from the perchlorinated fraction B. Comparison of the mass spectra of the perchlorinated crude grade quaterphenyl and the perchlorinated fraction B confirmed the tentative identification of polychlorinated quaterphenyls in fraction B.

The Food and Drug Administration is currently working to identify additional unidentified organic contaminants present in the "Yusho" rice oil.

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REFERENCES

ARMOUR, J.: J. Assoc. Offic. Anal. Chem. 56, 987 (1973).

FOOD AND DRUG ADMINISTRATION: Fed. Regist. 38, 18096 (July 6, 1973).

KURATSUNE, M., et al.: National Conference on Polychlorinated Biphenyls (Chicago, Ill., November 19-21, 1975); proceedings published as EPA 560/6-75-004, pp. 14-29 (March 1976).

FOOD AND DRUG ADMINISTRATION: Pesticide Analytical Manual, Vol. I, Section 211.14d (4/1/71 revision).

TANNER, J., AND M.H. FRIEDMAN: International Conference on Modern Trends in Activation Analysis, Munich, Germany, September 13-17, 1976, proceedings, pp. 344-351.

TSUKAMOTO, H., et al.: Fukuoka Acta Medica 60, 496 (1969).