Evidence of Tetrachlorodibenzofuran (TCDF) in Aroclor 1254[R], and the Urine of Rats Following Dietary Exposure to Aroclor 1254[R]*

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INTRODUCTION

Recent reports and past findings indicate that impurities found in some technical products may result in disease following exposure. Chloracne, X-disease and the chick edema factor are classic examples of disease resulting from exposure to chlorinated compounds, contaminants in the same, or both[1]. Chlorinated dibenzodioxins (CDD) and chlorinated dibenzofurans (CDF) have been the most implicated as contaminants of polychlorinated biphenyls (PCB).

VOS et al.[2] accounted for differences in the toxicities of three commercially available PCB preparations, namely: Phenoclor DPG, French; Clophen A60, German; and Aroclor 1260[R], U.S. by the presence of two polar compounds in the third fractions (25% Et_2O in hexane) of the French and German products, namely tetra and pentachlorodibenzofurans. Chlorinated dibenzofurans, including the tetrachlorodibenzofuran as well as pentachloronaphthalene were also identified in a Japanese PCB (Kanechlor 400)[3]. Neither was found in the U. S. product, Aroclor 1260[R].

This paper reports the details of the mass spectral findings of a tetrachloro compound with a molecular weight of 304 in the urine of rats following prolonged exposure to Aroclor 1254[R]. It also reports evidence in support of the presence of a similar compound in Aroclor 1254[R] itself.

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EXPERIMENTAL

In the first study sixteen-hour urine samples were collected from eight male Sherman strain rats. Seven had been on a dietary level of 100 ppm Aroclor 1254^{R} (between 13-5 mg/kg/day) for times varying from 4-58 days. One had been on a dietary level of 500 ppm Aroclor 1254^{R} (about 25 mg/kg/day) for 252 days. These rats were started on the experimental diet when they were about 45 days old. The total Aroclor consumption of all rats over the entire period of exposure up to the time the urine was collected was 3.0 grams. Urine averaged 11 mls per rat and was extracted separately with hexane at a pH of about 7 and eluted from micro silica gel columns with a 1:1 mixture of benzene:hexane according to the method of Curley et al.[4]. Samples were combined prior to mass spectral analysis.

In a second study, seven-day urine samples were collected from four female rats that had been fed Aroclor 1254^{R}, 100 ppm (7.5 mg/kg/day) for eight months. The pooled urine sample, 440 mls - pH 6.6, was extracted six times with 50 mls of diethyl ether-hexane (3:1). Each 50 mls of extract was centrifuged and the supernates combined. The extract was evaporated to 15 mls and partitioned with acetonitrile as described by Mills[5]. Prior to mass spectral analysis the sample was methylated using a procedure similar to that of Stanley[6].

Aroclor 1254^{R}, 1.7 grams, was dissolved in 300 mls of hexane. The chromatography column had an I.D. of approximately 34 mm and was filled with 180 grams of PR grade activated florisil. The column was pre-washed with hexane and the Aroclor standard was added in 300 mls hexane. The fractions and volumes collected are listed in Table I. Analysis of each fraction using Coulson Conductometry revealed a general pattern indicative of Aroclor 1254^{R}.

TABLE I

Elution of Aroclor 1254^{R} from Florisil

Elute	Vol. (mls)	Fraction
Hexane	1200	I
5% Et_2O in Hexane	1200	II
25% Et_2O in Hexane	200 mls each	III, IV, V, VI, VII, VIII
50% Et_2O in Hexane	500 mls	IX
50% Et_2O in Hexane	500 mls	X

RESULTS AND DISCUSSION

The mass spectrum resulting from the analysis of authenic 2,3,7,8 tetrachlorodibenzofuran (TCDF) is shown in figure 1. The molecular ion M^+/e 338 with a chlorine isotopic cluster indicative of 5 is the pentachlorodibenzofuran (PCDF) obtained as an impurity during the synthesis of TCDF. The low intensity ion at M^+/e 275 is the fragment resulting from the loss of 63 mass units from PCDF. The molecular

ion at M^+/e 304, TCDF, contains the base peak. The fragmentography of TCDF is characterized by the loss of 63 mass units (COCl) to yield the fragment at M^+/e 241 and subsequent loss of 70 mass units (2Cl) to yield M^+/e 171. Doubly charged ions were observed at M^{+2}/e 152 and M^{+2}/e 120.5 with isotopic clusters synonomous with those of their singly charged counterparts at M^+/e 304 and M^+/e 241 respectively. The characteristic loss of COCl has been observed in the fragmentation of similarly structured compounds, namely the chlorinated dioxins and higher chlorinated dibenzofurans Curley et al.[7].

The urine spectrum from the first study (Fig 2A) shows the presence of pentachlorobiphenyl (M^+/e 324) and the fragment resulting from the loss of 70 mass units to yield M^+/e 254. Low intensity ions at M^+/e 288 and M^+/e 290 were observed. The molecular ion at M^+/e 304 contains an isotopic cluster indicative of 4 Cl with one major fragment at M^+/e 235 indicative of the loss of 2 Cl. Observation of the spectrum below M^+/e 230 revealed that it was attributed entirely to PCB's.

Fraction V from the Aroclor 1254^R florisil elution (Fig 2B) contains the hexachlorobiphenyl (M^+/e 358), pentachlorobiphenyl (M^+/e 324) in addition to tri and tetrachlorobiphenyl fragments (M^+/e 288 and M^+/e 253) resulting from the loss of 2 Cl from 358 and 324 respectively. Also present in the spectrum is the molecular ion M^+/e 304 with an isotopic cluster indicative of 4 Cl. This ion compares favorably with TCDF. The low intensity of the ion at 304 precluded observance of any fragmentation.

Figure 2C shows the spectrum produced by urine that had been methylated. The spectrum indicates the presence of a monomethoxy derivative of pentachlorobiphenyl (M^+/e 354) with major fragment ions indicative of: (1) loss of methyl and carbon monoxide (M^+-43) to yield M^+/e 311 and (2) subsequent loss of methyl and carbon monoxide and two chlorines (M^+-113) to yield M^+/e 241. This spectrum also contains a molecular ion with an isotopic cluster indicative of four chlorines at M^+/e 304.

Mass spectral evidence does not establish unequivocally the presence of TCDF. However, it can be said that the molecular ion, 304, is not the result of fragmentation of a higher molecular weight component of the isomeric commercial Aroclor 1254^R mixture. There is a noticeable difference in the relative intensities of the molecular ions, M^+/e 304, in the urine and standard Aroclor 1254^R. The amount of Aroclor represented in Fraction V is 1.7 grams, while the urine represents 3.0 grams consumed over an extended period. The urine sample enrichment in the analyzer tube was low necessitating a greatly amplified normalized spectrum.

Hutzinger et al.[8,9], have reported the presence of hydroxylated biphenyls in urine resulting from metabolism in addition to the presence of oxygen derivatives resulting from irradiation of Aroclor 1254^R films; spectra in both instances show the presence of M^+/e 306 with an isotopic pattern indicative of four chlorines.

This author and co-workers have observed a molecular ion matching the 306 species reported by Hutzinger, however its fragmentation has been characterized by the predominance of the

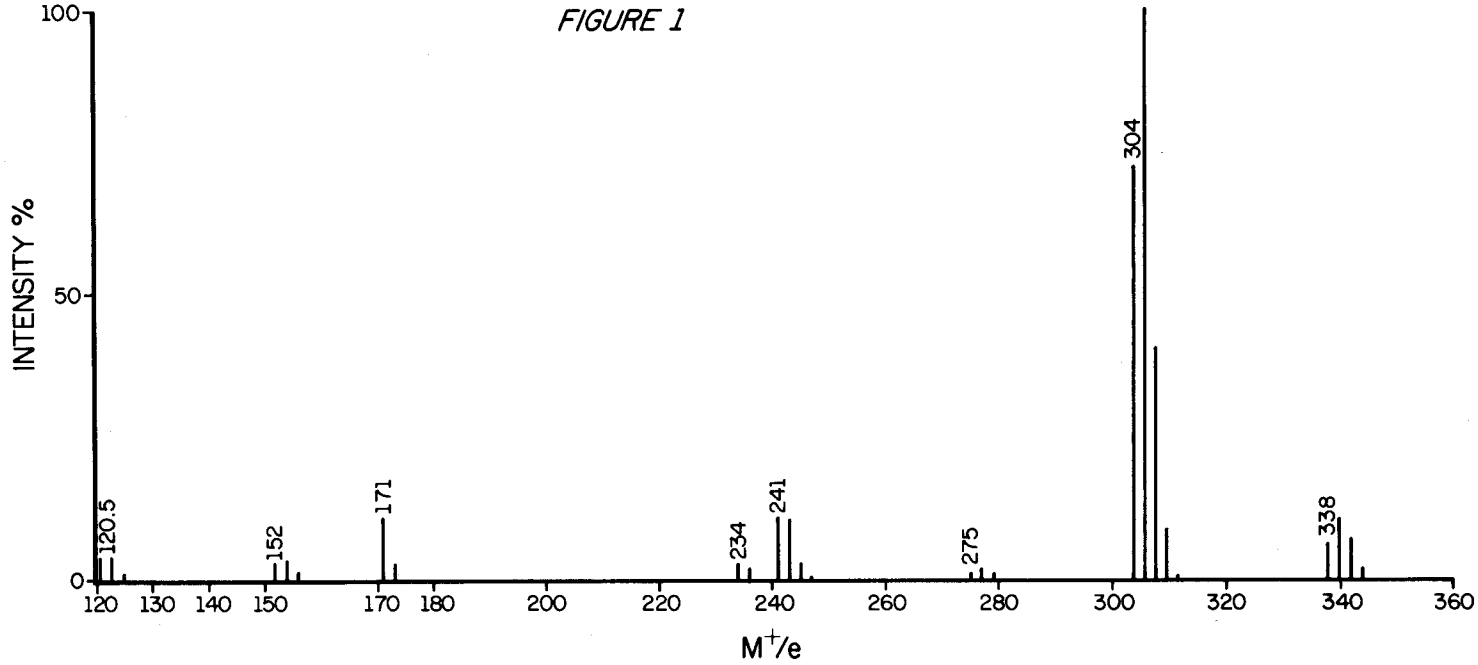

Figure 1 Mass Spectrum resulting from the direct probe analysis, at 30°C and 70 eV, of authenicated 2,3,7,8 tetrachlorodibenzofuran; see Figure 2A for other conditions.

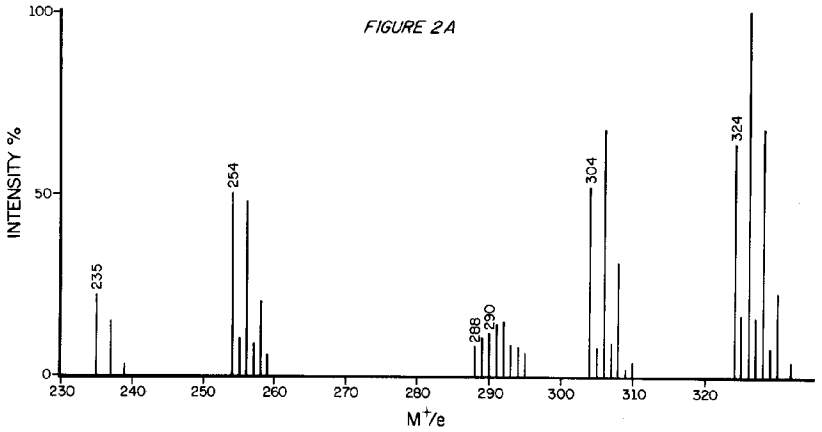

Figure 2A Mass spectrum resulting from GC-MS analysis of 88 mls of rat urine. LKB 9000 GC-MS, mass marker ±0.3 mass unit. GC column temperature, 209°C; flash heater 235°C; glass coiled column, 6'x1/4" 1.5 OV-17/1.95 QF-1 on 60/80 mesh chromosorb "W" H.P., A.W., DMCS; carrier gas (He) 30 psi and 45 cc/min.; separator, 320°C; source 290°C; energy, 70 eV; accelerating voltage, 3.5 KV; trap current, 60 uA; box current, 50 uA; leak current, 8 uA.

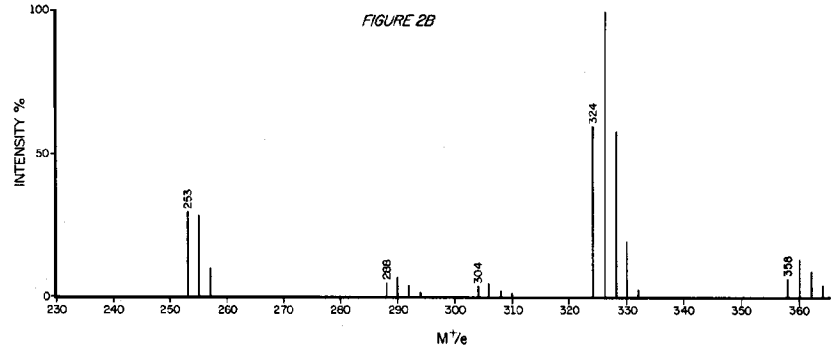

Figure 2B Mass spectrum resulting from GC-MS analysis of Fraction V 25% Et_2O/Hex of Aroclor 1254®. LKB 9000 GC-MS, other conditions see fig. 2A. Instrument calibrated with PFK.

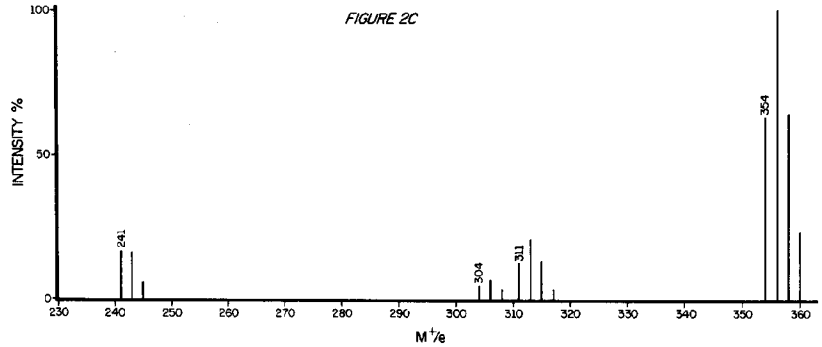

Figure 2C Mass spectrum of 220 mls urine following methylation with diazomethane. Mass spectral conditions see fig. 2A.

fragment resulting from the loss of 70 mass units.

The molecular ion M^+/e 304 was also present in methylated urine obtained from 4 female rats that had consumed Aroclor 1254^R for eight months (Fig 2C). This finding indicates that a 304 tetrachloro component is present in the urine and at a trace level in the analyzed commercial PCB preparation.

A trace level of the 304 tetrachloro component was also present in the analyzed commercial PCB preparation. One would have to consider the possibility of such a compound existing in the PCB preparation from a review of the purification process. This process consists of distillation of the crude material at reduced pressures (about 50 mm Hg) and elevated temperatures (150°C-300°C) in the presence of a few tenths of 1% of lime or sodium hydroxide, Papageorge[10], Hubbard[11], and could lead to hydroxylation and the subsequent loss of HCl could lead to a dibenzofuran derivative.

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