A Comparison of Analytical Methods for Chlorodibenzo-*p*-dioxins in Pentachlorophenol

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The chlorodibenzo-*p*-dioxin (CDD) contamination of the same commercial pentachlorophenol (PCP) was determined by four methods described in the literature. The CDD levels were estimated by electron capture–gas chromatography (EC–GC). The presence of CDD, chlorodibenzofuran (CDF), and chlorodiphenyl ether (CDE) was confirmed by gas chromatography–mass spectrometry (GC–MS). Hydroxychlorodiphenyl ethers were also found. The results show wide variations as a function of the analytical method.

Pentachlorophenol (PCP) has been widely used as a fungicide, slimicide, and wood preservative. The contamination of PCP by chlorodibenzo-*p*-dioxin (CDD) has been reported (Firestone et al., 1972; Jensen and Renberg, 1972; Villanueva et al., 1973; Woolson et al., 1972; Plimmer et al., 1973). The hexa-, hepta-, and octa-CDD are the ones most commonly found in PCP. Other structurally related compounds, such as chlorodiphenyl ether (CDE) and chlorodibenzofuran (CDF), have been found in the extracts of PCP. These compounds have similar retention characteristics to CDD. Therefore, GC–MS is used to confirm the presence of CDD and to substantiate the presence of other chlorinated moieties with similar retention characteristics.

In addition to the CDD, a CDD precursor, hydroxychlorodiphenyl ether (pre-CDD), has also been found in PCP (Jensen and Renberg, 1972; Rappe and Nilsson, 1972). If the pre-CDD is not removed from the sample before gas chromatographic analysis, the heat of the injection block may cause cyclization of the pre-CDD to the corresponding CDD. This would give erroneously high results for CDD actually present in the sample. Any method used to analyze for CDD must eliminate the possibility of conversion of the pre-CDD to CDD.

This paper reports a comparison of four published analytical methods for CDD in PCP. For all methods only one lot of PCP (technical grade) was analyzed in order to keep the CDD level constant. Thus, variation in results could be attributed to the analytical method employed. Duplicate samples were analyzed by each method. These methods are designated as follows: (1) Firestone, 1971; Firestone et al., 1972; (2) Jensen and Renberg, 1972; (3) Crummet, 1973 (a modification of the procedure used in this paper has been published: Crummett and Stehl, 1973); and (4) Rappe and Nilsson, 1972. The Firestone method involves the extraction of the nonacidic material in the PCP sample with petroleum ether, concentration of this extract, and then addition of the extract to an alumina column. The eluate from the column was shaken with concentrated sulfuric acid, passed through a column of sodium sulfate and sodium carbonate, and concentrated for analysis by EC–GC. The Jensen method involved the extraction of the nonacidic material in the PCP sample with ether–hexane (1:1), concentration of the extract, treatment with diazomethane, and concentration for EC–GC analysis. With the Crummett method the acidic components were removed by means of an ion exchange column. Ethanol–chloroform (1:3) was used as a solvent and eluent. The eluate was concentrated and analyzed by EC–GC. In the Rappe method PCP was treated with diazomethane and then analyzed by EC–GC.

In all methods the CDD levels were estimated by EC–GC using the peak height technique and available CDD standards. GC–MS was employed to confirm the presence of the CDD and to identify other contaminants in the PCP.

EXPERIMENTAL SECTION

A Varian 2100 gas chromatograph with a tritium detector was equipped with a 6 ft × 0.25 in. o.d. U-shaped glass column packed with 3% OV-1 on 80–100 mesh Supelcoport. The column temperature was 220°; detector, 200°; and injector, 235°. The nitrogen flow was 65 ml/min at 52 psi.

Two CDD standards were used for quantitation of the CDD levels in PCP. Octa-CDD was purchased from Analabs, Inc., North Haven, Conn. Hexa-CDD was synthesized according to the method of Pohland and Yang (1972). The hexa-CDD (three isomers) was purified by sublimation and then by preparative GC. A Varian Aerograph 1520 with a flame ionization detector was used with a 6 ft × 0.25 in. o.d. coiled aluminum column packed with 1.5%/1.95% OV-17/SP-2401 on 80–100 mesh Supelcoport. The column temperature was 220°; injector, 210°; and detector, 205°. In the preparation of hexa-CDD, some hepta-CDD was also formed but was not of sufficient purity to be used for quantitation; however, it was used to establish retention times. An octa-CDF standard was purchased from Analabs, Inc.

Mass spectral analysis was accomplished on an LKB 9000 equipped with a mass marker (±0.3 mass unit) and interfaced to a 10 ft × 0.25 in. o.d. coiled Pyrex glass column packed with 3% SE-30 on 60–80 Chromosorb G (DMCS treated, acid washed). The column temperature was 230°. The helium flow rate was 70 ml/min at 12 psi. Under these conditions the octa-CDD standard had a retention time of 72 min.

The amount of octa-CDD recovered from PCP by each method was determined by spiking duplicate samples of pure PCP (99+%) with octa-CDD at levels approximating the CDD levels found in technical PCP. The octa-CDD levels in the pure PCP were too low to contribute to the octa-CDD recovery. Recovery studies on hexa-CDD and hepta-CDD were not done because of an insufficient amount of hexa-CDD and the impurity of hepta-CDD.

RESULTS AND DISCUSSION

The levels of CDD in PCP obtained by the four methods of analysis, as determined by EC–GC, are listed in Table I. The gas chromatograms resulting from each method are shown in Figures 1A–D. No correction was made for recovery. The levels of hepta-CDD were estimated from the octa-CDD standard. [The assumption has been made that

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Table I. EC-GC Results of PCP Analysis

Method	CDD levels, ppm		
	Hexa-CDD	Hepta-CDD[a]	Octa-CDD
Firestone	109	439	204
Jensen	551	903	1517
Crummett	429	962	1255
Rappe		3798	8042

[a] Based on octa-CDD standard.

Table II. Percent Recovery of Octa-CDD

Method	Level of octa-CDD, ppm		
	100	1000	10,000
Firestone	57	47	
Jensen	96	65	
Crummett	99	93	
Rappe	77	80	105

the response factor for hepta-CDD is very close to the response factor for octa-CDD. In this study the ratio of response factors for octa-CDD standard to hexa-CDD standard is 1.3.] The Jensen and Crummett methods agree fairly well. The Firestone method gives somewhat lower results. The Rappe method, which has no cleanup step, gives extremely high results.

The recovery of octa-CDD by each method is given in Table II. The highest recovery was obtained by the Crummett method and the lowest by the Firestone method.

The Crummett and Jensen methods gave the best recovery values, while the Crummett and Rappe methods were the simplest methods. The Rappe method, however, was found unacceptable because the values appeared unreasonably high in comparison to those obtained with the other methods. When the Rappe solution was analyzed on a GC with a Coulson conductometric detector, the results were in the same range as found by EC-GC. Also, the hexa-CDD peak was totally obscured by tailing of the methylpentachlorophenate (Figure 1D). This method serves as a check for the presence of hepta-CDD and octa-CDD and their corresponding pre-CDD. The low recovery of the Firestone method was expected, since many steps were involved before EC-GC analysis. Firestone et al. (1972) reported an average recovery of 32.7% for tetra-CDD from trichlorophenol spiked at 4 ppm.

Analysis by GC-MS confirmed the presence of hexa-, hepta-, and octa-CDD and identified the other peaks found by EC-GC (Figure 1). These were hexa-, hepta-, and octa-CDE and hexa-, hepta-, and octa-CDF. The methylated precursors of hepta-CDD and octa-CDD were identified in

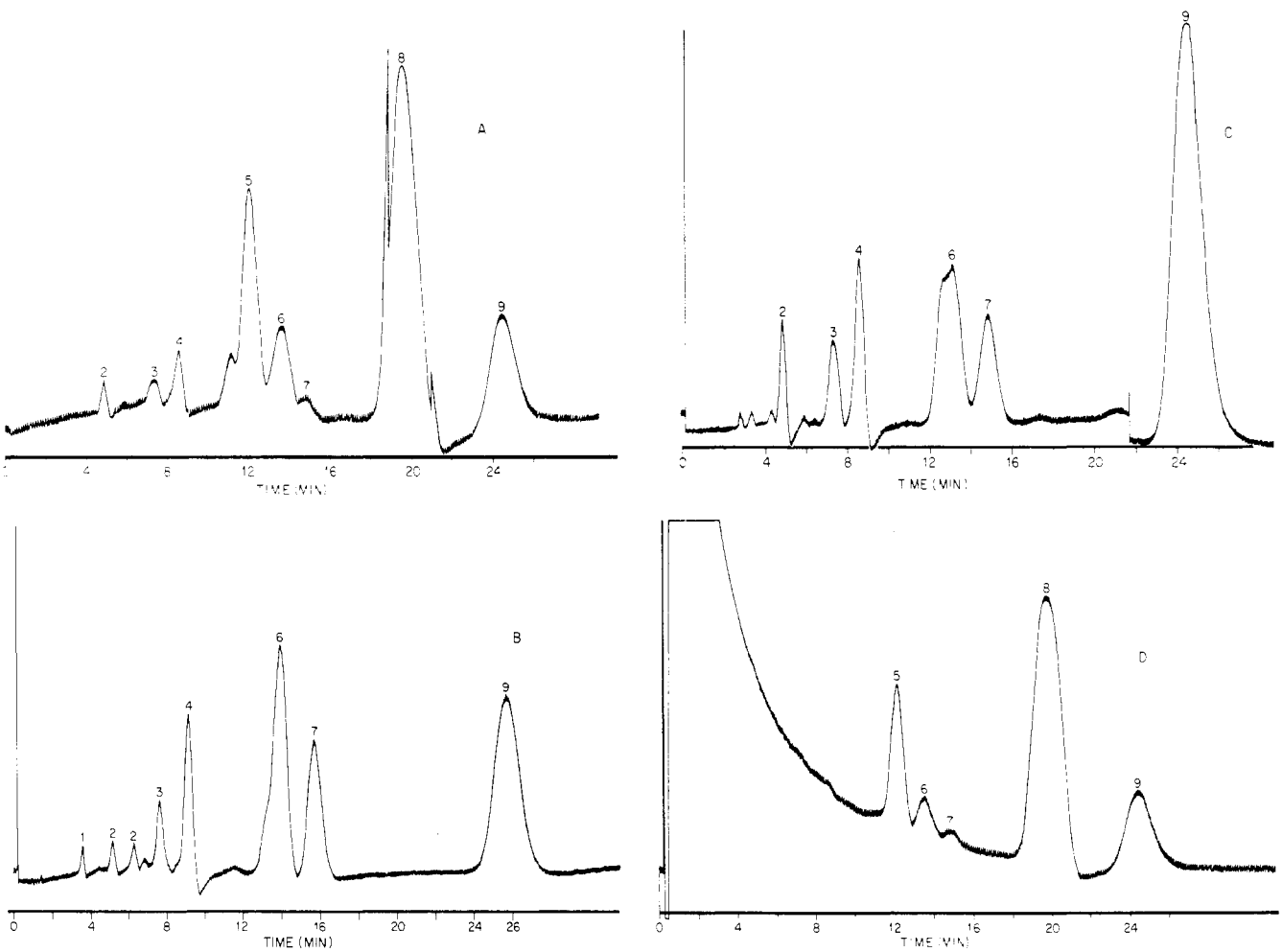

Figure 1. Gas chromatograms resulting from PCP analysis after treatment (A) by Jensen method, (B) by Firestone method, (C) by Crummett method, and (D) by Rappe method: (1) hexa-CDE; (2) hepta-CDE; (3) hexa-CDF and octa-CDE; (4) hexa-CDD and octa-CDE; (5) methylated pre-hepta-CDD; (6) hepta-CDD and hepta-CDF; (7) hepta-CDD; (8) methylated pre-octa-CDD; (9) octa-CDD and octa-CDF.

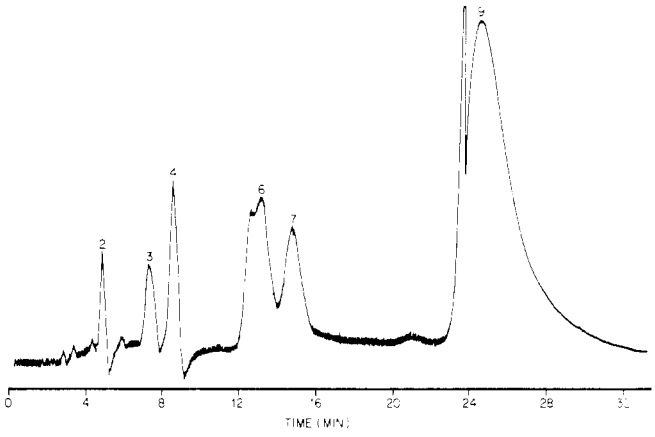

Figure 2. Gas chromatogram of extract from PCP by Jensen method before methylation. For peak identification, see Figure 1.

the Jensen and Rappe methods. The octa-CDE eluted with hexa-CDD, hepta-CDF eluted with hepta-CDD, and octa-CDF eluted with octa-CDD.

The technical PCP was analyzed by EC–GC before and after the methylation steps of Rappe and Jensen methods. If pre-CDD were present, conversion of the pre-CDD to the CDD during EC–GC would be expected; thus, the CDD level would be higher in the unmethylated sample than in the methylated sample. In previously reported results (Jensen and Renberg, 1972; Rappe and Nilsson, 1972) and in results found in this laboratory on other PCP preparations, the CDD levels were much higher before methylation than after methylation. In the technical PCP analyzed here, however, the octa-CDD and hepta-CDD levels were only slightly higher in the unmethylated sample, Figure 2, than in the methylated sample, Figure 1A. The levels were 1.4 and 1.2, respectively.

Two large new peaks appeared in the methylated sample (Figure 1A). By GC–MS analysis, these new peaks were identified as the methylated pre-CDD which convert to octa-CDD and hepta-CDD. The failure of most of the pre-CDD in the unmethylated sample to convert to the CDD suggests that this technical PCP may contain most of the pre-CDD in the iso pre-CDD form.

Jensen has suggested that the iso pre-CDD is an isomeric form of the pre-CDD in which the hydroxy group is in position 1 or 2 on the ring. Ring closure to the CDD is thereby prevented.

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Gas Chromatographic–Mass Spectrometric Studies of Ethoxyquin in Some Organic Solvents. I

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The stability of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin, EMQ) was studied in *n*-hexane and chloroform with emphasis on the color changes of the solutions and the quantitative changes during 1 month of storage in the absence of light. Visual observations, gas–liquid chromatography (GLC), and GLC combined with mass spectrometry (MS) were used as the methods of analysis. The antioxidant was found to be extremely labile on exposure to light, and in chloroform solutions an increase of color intensity was observed together with a 35–70% loss of GLC measurable EMQ, the tenfold dilute solutions (0.1 mg/ml) being the least stable. The ethoxyquin dissolved in *n*-hexane, however, was found to remain unchanged even after the storage period. In conclusion, *n*-hexane is therefore recommended as the solvent for use in analytical work and for extractions from biological systems containing ethoxyquin. GLC using a 3% SE-30 column operated at 160° has been found to be suitable for quantization of EMQ when residues are to be determined in food products for example. The mass spectra of the GLC peaks were examined for characteristic fragmentation patterns.

6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, EMQ (ethoxyquin), is a remarkably effective antioxidant and is gaining increased application as a feed additive (Monsanto Chemical Co., 1961; Opstvedt et al., 1970, 1971). The necessity of understanding the chemistry of feed and food additives, and the fate of these substances in various biological systems, require that the compounds in question be studied under defined conditions, and that procedures for the exact determination of the additives and their breakdown products are available. In this paper we report some investigations on the choice of solvent for work with ethoxyquin

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