Determination of Pentachlorophenol, Hexachlorodibenzo-*p*-dioxin, and Octachlorodibenzo-*p*-dioxin in Bovine Milk

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Methods are described for determining pentachlorophenol (PCP), hexachlorodibenzo-*p*-dioxin (HCDD), octachlorodibenzo-*p*-dioxin (OCDD) in bovine milk. The PCP method consists of sulfuric acid digestion, silica gel column chromatography, methylation, alumina column chromatography, and detection with electron capture–gas chromatography (EC–GC). The chlorodioxin procedure consists of extraction from basic solution, acid wash, silica gel and alumina column chromatography, and detection by EC–GC and/or gas chromatography–mass spectrometry (GC–MS). Recoveries for PCP, HCDD, and OCDD were 80, 80, and 100%, respectively. Samples of some quarantined milk from the State of Michigan gave no detectable signal for PCP, HCDD, and OCDD at the detection limits of 10–15, 0.025, and 0.05 ppb, respectively.

Pentachlorophenol (PCP) is an antimicrobial agent that is widely used as a wood preservative. Commercial PCP contains trace amounts of chlorodioxins, some of which have been shown to be toxic (Schwetz et al., 1973; Flick et al., 1972). In March 1977, the Michigan Department of Agriculture quarantined several dairy herds because of possible milk contamination by PCP and/or chlorodibenzo-*p*-dioxins. Methods were developed to determine if milk from these herds was contaminated.

Because of the high sensitivity available, PCP has been determined using electron capture–gas chromatography (EC–GC) with a variety of derivatizing reagents, i.e., trimethylchlorosilane (Stark, 1969), acetic anhydride (Rudling, 1970; Zitko et al., 1974; Chau and Coburn, 1974), diazomethane (Renberg, 1974), and diazoethane (Gee et al., 1974). Recently, negative ion chemical ionization mass spectrometry has been used for this analysis (Dougherty and Piotrowska, 1976a,b).

Chlorodibenzo-*p*-dioxins have been determined in commercial PCP materials by liquid chromatography (Pfeiffer, 1976) and gas chromatography–mass spectrometry (Blaser et al., 1976). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, a possible component in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), has been determined in bovine milk at the low part-per-trillion level (Mahle et al., 1977; Shadoff and Hummel, 1975) but nothing has been reported on HCDD and OCDD in milk. This paper describes methods for determining PCP, HCDD, and OCDD in bovine milk.

EXPERIMENTAL SECTION

Safety. All procedures using benzene should be carried out in a ventilated hood. The EXR 101 reagent and diazomethane are toxic, skin sensitizers, and explosive. Care should be taken to avoid skin contact with the PCP, HCDD, and OCDD standards and standard solutions. Gloves should be worn when the standards are being prepared.

Gas Chromatographic Analyses. The samples were analyzed on a Hewlett-Packard Model 5713A gas chromatograph with an electron-capture detector (^{63}Ni). For the determination of methylated pentachlorophenol a 300 × 0.2 cm (i.d.) glass column was packed with 0.5% w/w Silar 10C on 80–100 mesh Chromosorb W-AW. This support was deactivated before coating with liquid phase by a procedure similar to that described by Karasek and Hill (1975). The column temperature was programmed from 100 to 200 °C at 8 °C/min, the nitrogen carrier flow rate was 34 mL/min, and the injection port and detector temperatures were 200 and 300 °C, respectively.

HCDD and OCDD analyses were performed on a 180 × 0.2 cm (i.d.) glass column packed with 3% OV-3 on 100/120 mesh Chromosorb W HP. Column temperature was 280 °C while injection port and detector were held at 300 and 350 °C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min.

Gas Chromatography–Mass Spectrometry. The samples were run on an AEI MS-30 gas chromatograph–mass spectrometer (GC–MS) using either a 180-cm glass column packed with 0.1% OV-105 on 100/120 mesh GLC-100 glass beads or a 90-cm glass column packed with 3% OV-3 on 100/120 mesh Gas-Chrom Z. The spectrometer signal was monitored by a Nicolet 1072 signal averager. The sweep is produced by allowing a capacitor to charge in the feedback circuit of the electric sector power supply producing a linear voltage ramp and thus a linear mass sweep. The details of this have been previously described (Shadoff and Hummel, 1978). The spectrometer was set to sweep a narrow mass range at m/e 390 for HCDD or m/e 460 for OCDD at a resolving power of 3000. The silicone membrane separator was run at 215 °C. Other spectrometer conditions include: ion source temperature, 250 °C, electron energy, 70 eV; and trap current, 300 μA.

In order to obtain a real-time ion current display, a PAR Model CW-1 boxcar integrator was set to monitor and display a portion of the mass sweep. The boxcar integrator was triggered by the Nicolet 1072 +GATE OUT signal. The delay and gate width were adjusted to display only that signal due to mass region for the HCDD. Thus both an accumulated mass scan and a mass chromatogram can be obtained simultaneously. A typical output using these techniques for a 5-μL injection of a 10 ppb HCDD standard (50 pg) is shown in Figure 1. The higher mass spectral resolution separates the HCDD from the adjacent gas chromatographic column bleed and other sample matrix m/e peaks. This is in contrast to the low-resolution mass spectral method in which the small HCDD peak would be superimposed on the large column background.

Reagents. Benzene, hexane (Mallinkrodt Nanograde), ethanol (MCB Pesticidequality), methylene chloride, carbon tetrachloride (Burdick and Jackson, Distilled-in-Glass), sodium hydroxide, and sulfuric acid (J. T. Baker Analyzed Reagent) were used without further purification. Diazomethane was prepared from EXR101 (*N*,*N*-dinitroso-*N*,*N*-dimethylterephthalamide) obtained from DuPont Chemical Company. BioSil A silica gel, 100/120 mesh, and Acid Alumina AG4, 100/120 mesh (Bio-Rad Laboratories), were used without purification for pentachlorophenol analysis. The BioSil A was deactivated with

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Table I. Results of Analysis of Bovine Milk[a]

sample no.		Michigan county	PCP concn, ppb[b]	HCDD concn, ppb	OCDD concn, ppb
D-00601	subsample C	Barry	ND (15)	ND (0.025)	ND (0.050)
D-02602	subsample B	Shiawassee	ND (15)	ND (0.025)	ND (0.050)
D-05327	subsample A	Cass	ND (15)	ND (0.025)	ND (0.050)
D-05652	subsample 3	Arenac	ND (15)	ND (0.025)	ND (0.050)
D-05653	subsample C	Arenac	ND (15)	ND (0.025)	ND (0.050)
D-05654	subsample 1	Arenac	ND (10)	ND (0.025)	ND (0.050)
D-05654	subsample 2	Arenac	ND (10)	ND (0.025)	ND (0.050)
D-05654	subsample 3	Arenac	ND (10)	ND (0.025)	ND (0.050)

[a] Analyses by electron capture-gas chromatography. [b] ppb = ng/g. [c] ND = not detected at the limit of detection given in parentheses.

Table II. Effect of Potassium Hydroxide Concentration, Time, and Temperatures on Chlorodioxin Stability

			HCDD		OCDD	
temperature digestion, °C	digestion time, h	percent KOH concn	initial concn, ppb	percent decomposition	initial concn, ppb	percent decomposition
22	24	20	1	14	1	44
35	24	20	1	54	1	72
60	24	20	1	72	1	>95
80	24	20	1	>95	1	>95
22	1	4	0.1[a]	10[b]	0.1[a]	10[b]
22	2	4	0.1	10[b]	0.1	10[b]

[a] Lower detection limits are possible because no sample matrix is present. [b] These values are reported to one significant figure.

7% (w/w) water and the Acid Alumina with 1% (w/w) water. Alumina (Fisher A540, 80/200 mesh) and silica gel (Davison 923, 100/120 mesh) were used in the chlorodioxin analysis after purification as described below.

Thirty-gram portions of alumina and silica gel in 25 × 80 mm paper extraction thimbles (Whatman) were Soxhlet extracted with methylene chloride for 16 h. The alumina had been activated at 130 °C for 12 h before extraction. Residual methylene chloride was removed under vacuum.

The purities of all reagents were checked by the analysis of blanks with each set of samples.

Sample Collection and History. The samples were collected in polyethylene bags by Michigan Department of Agriculture personnel from areas noted in Table I. These samples were refrigerated during transport to the Dow Chemical Company (Midland, Mich.) and then frozen until analyzed.

No data were available on the exposure of the cows to pentachlorophenol. The PCP used to treat the wood to which the animals were exposed was apparently produced by various manufacturers. It is not known if technical or purified grades of PCP were used.

Sample Preparation for Chlorodioxin Analysis. The clean-up procedure was essentially that of Hummel (1977) and Mahle et al. (1977). Five-gram samples were made basic with 2 mL of 40% potassium hydroxide and 10 mL of ethanol. The material was then immediately extracted with hexane and the extract was washed with concentrated sulfuric acid. The residue was further cleaned up with silica gel and alumina column chromatography. The effect of potassium hydroxide digestion concentration, temperature, and time on chlorodioxin decomposition was studied; and the results are shown in Table II. As can be seen from the data, lengthy periods of digestion at elevated temperatures will drastically reduce HCDD and the OCDD concentrations. This phenomenon was also noted by Firestone (1977). To avoid this problem, the milk was made alkaline with a smaller amount of potassium hydroxide.

Sample Preparation for Pentachlorophenol Analysis. A 5-g portion of milk was weighed into a 60-mL glass bottle containing 25 mL of 1:1 (v/v) H_2SO_4–water and 10 mL of 20% (v/v) benzene–hexane and placed in a water bath at 35 °C for 8 h. The mixture was transferred to a 60-mL separatory funnel, and the phases were separated. The aqueous phase was reextracted three times with 5 mL of 20% (v/v) benzene–hexane. All of the

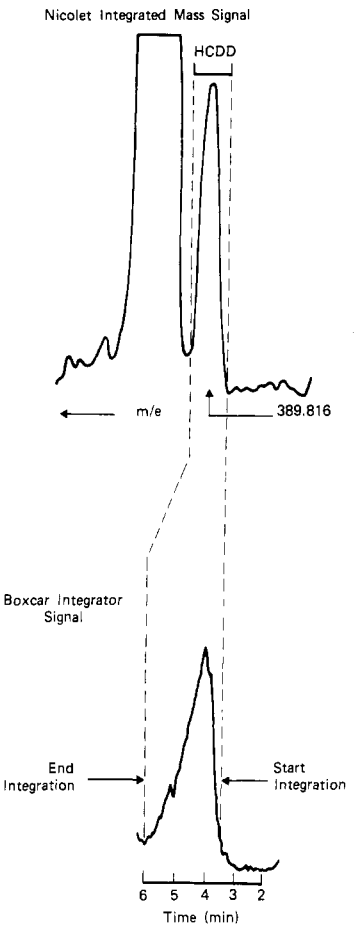

Figure 1. Typical signals from simultaneous determination of integrated ion current vs. m/e and ion current intensity vs. time for a 50-pg HCDD standard.

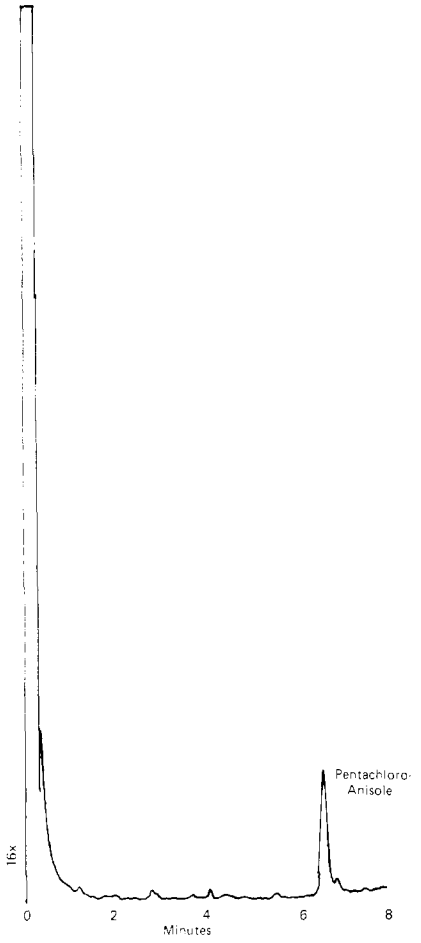

Figure 2. Electron capture–gas chromatogram of extract of milk fortified with 36 ppb pentachlorophenol.

extracts were combined, and the volume was adjusted to 25 mL. A 10-mL aliquot of the combined extract containing the equivalent of 2 g of milk was added to the top of a 50 × 5 mm column of BioSil A. The column was then eluted with 10 mL of benzene; and both eluent fractions (20% benzene–hexane and 100% benzene) were combined. One milliliter of methanol was added to the combined extracts and methylated with diazomethane to form pentachloroanisole. The excess diazomethane was removed under a stream of nitrogen and the solvent evaporated to 1 mL in a Kuderna-Danish evaporative concentrator. Four milliliters of hexane was added to the concentrator tube, and this solution was transferred onto a 50 × 5 mm column of Acid Alumina AG4. The column effluent was collected in a 10-mL Kuderna-Danish concentration tube and an additional 5 mL of 20% (v/v) benzene in hexane added to the column. Both fractions were combined and concentrated to 1 mL as before and analyzed by gas chromatography.

RESULTS AND DISCUSSION

Five-gram portions of control milk that had been analyzed and found to contain no detectable HCDD, OCDD, or pentachlorophenol were fortified with either HCDD and OCDD to concentrations of 50 pg/g or pentachlorophenol at 36 ng/g. Small volumes (2.5 μL of a 100 ng/mL of benzene solution for HCDD and OCDD or 25 μL of a 7.2 μg/mL solution for PCP) were mixed with ethanol and added to the milk before the sample digestion. Results of recovery experiments are shown in Table III. The recoveries for PCP using this procedure are consistent with those obtained in our laboratory from a variety of other biological matrices.

Table III. Results of Recovery Experiments from Fortified Milk

compound	concn added, ppb	concn found, ppb	% recov.
pentachlorophenol	36	27	75
	36	29	81
	36	30	83
hexachlorodibenzo-*p*-dioxin	0.05	0.04	80
	0.05	0.04	80
octachlorodibenzo-*p*-dioxin	0.05	0.05	100
	0.05	0.05	100

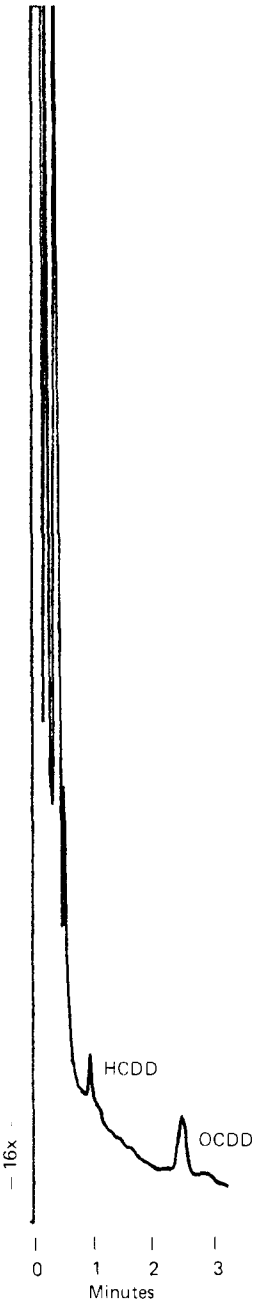

Figure 3. Electron capture–gas chromatogram of extract of milk fortified with 50 ppt each of HCDD and OCDD.

The limit of detection was calculated as 2.5 times either the peak-to-peak noise or the reagent blank signal. The EC–GC results of this study are shown in Table I with limits of detection in parentheses. PCP, HCDD, and OCDD were not detected in any of the samples. The chlorodioxin detection limits are average values for HCDD concentrations in the range of 20–30 ppt and 40–60 ppt

for OCDD. This variation is similar to that observed by Zitko (1975) for other organochlorine compounds in biological samples that were collected during a carefully controlled sampling program.

During our initial attempts, apparent contamination was observed in both reagent blanks and sample extracts. A previous author (Wilkinson, 1975) has also observed gas chromatographic interferences in the determination of OCDD. Interferences produced apparent positive results. For example, a response corresponding to 40 ppt of HCDD was observed in the extract of sample D-05654 subsample 3. After purification of the liquid chromatographic adsorbents as described in the Experimental Section, the interferences were significantly reduced and the apparent positive result could not be reproduced. All three subsamples were analyzed to insure the initial analysis was indeed a false positive result. Reagent blanks were analyzed concurrently with each set of samples to insure that this interference did not reoccur. Control milk samples were analyzed for PCP, HCDD, and OCDD. In each case the controls were identical to the reagent blanks.

By analyzing the milk for residues of pentachlorophenol, HCDD, and OCDD, we had hoped to provide data on the types of impurities present in PCP. Tetrachlorophenols would be expected to bioconcentrate less than pentachlorophenol; and since they are present in the starting material at lower concentrations, would not be expected in the milk if PCP is not observed. Chlorinated dibenzofurans have been observed in PCP at concentrations comparable to the dioxins (Buser and Bosshardt, 1976). Although chlorinated dibenzofurans and heptachlorodibenzodioxins were not quantitatively determined, their presence would have been observed in the EC–GC chromatograms if present at concentrations similar to HCDD and OCDD.

The clean-up procedure described, coupled with EC–GC, provides a suitable screening technique for the determination of PCP, HCDD, and OCDD in bovine milk. As shown in Figures 2 and 3, the electron-capture gas chromatograms are relatively free of interfering components. In the event that a positive HCDD or OCDD response or significant interference is observed, confirmation of identity by GC–MS with comparable detection limits is required.

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A Simple Apparatus and Quantitative Method for Determining the Persistence of Pesticides in Soil

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A simple, noncumbersome apparatus and full supporting methodologies have been developed for quantitative studies on the rate and extent of degradation of ^{14}C-labeled pesticides in soil under aerobic conditions. Evolved $^{14}CO_2$ is monitored periodically using interchangeable Drierite–Ascarite–polyurethane towers with analysis by liquid scintillation counting after release of $^{14}CO_2$ from Ascarite with acid and retrapping. Aerobic conditions are maintained by diffusion through the tower while volatile organics are trapped in the polyurethane plug. Using multiple incubation flasks with a given soil and ^{14}C pesticide, the rate of degradation can be determined by periodic analysis of a whole flask. The overall accountability of the radioactivity using this methodology is nearly quantitative as shown by control experiments and by the results of many aerobic metabolism studies.

Two basic types of incubation systems have been developed and utilized to measure the rate and extent of degradation of pesticides and other ^{14}C-labeled compounds. The merits of these systems, as discussed by Parr and Smith (1969) and Bartha and Pramer (1965), have been summarized below. The open system employing a manifold assembly has been described by Parr and Smith

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