Determination of Polychlorodibenzo-*p*-dioxins and Polychlorodibenzofurans in Commercial Gelatins by Gas–Liquid Chromatography

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Residues of polychlorodibenzo-*p*-dioxins (dioxins) and polychlorodibenzofurans (furans) were found in commercial gelatin samples. The presence of dioxins and furans in the gelatin samples might have resulted from contamination of hides used as raw materials for gelatin manufacture by commercial pentachlorophenols (PCP), which may contain dioxins and furans. Fifteen-gram samples, after extraction and cleanup, were examined by electron-capture gas–liquid chromatography (EC–GLC). Recoveries of a number of dioxins and furans from fortified gelatin samples averaged 89 to 103%, with the exceptions of octachlorofuran (39%) and octachlorodioxin (70%). Fourteen of 15 commercial gelatins examined contained hexa-, hepta-, and octachlorodioxins at levels from 0.1 to 28 ppb of total dioxins. Levels of individual dioxins as low as 0.01 ppb were detected. Three bulk gelatins of Mexican manufacture contained from 24 to 28 ppb of total dioxins. Levels up to 0.4 ppb of heptachlorofuran and 0.4 ppb octachlorofuran were found in ten of the gelatins. Photodechlorination appeared to be useful for confirmation of octachlorodioxins in the samples. The presence of hexa-, hepta-, and octachlorodioxin and penta-, hexa-, and heptachlorofuran in one of the Mexican samples was confirmed with GLC–mass spectrometry.

Polychlorodibenzo-*p*-dioxins (dioxins) are formed in condensation reactions from ortho-substituted chlorophenoxy radicals (Kulka, 1961) or anions (Pohland and Yang, 1972). Dioxins, along with related compounds such as polychlorinated dibenzofurans (furans), were found in pentachlorophenol (PCP) and other commercial chlorophenols (Firestone et al., 1972). In addition, dioxins have been found in foodgrade oleic acids, emulsifiers prepared from the oleic acids, and fleshing greases isolated from hides treated with commercial PCP. The presence of dioxins in food materials is of concern because they are fat-soluble, resist biological degradation, and tend to accumulate in the food chain. Many dioxins produce severe toxicological responses (Flick et al., 1972; Harris et al., 1973; Schwetz et al., 1973) and cause embryonic deformities in chicks (Flick et al., 1965) and mice (Neubert et al., 1973). Little is known about the toxicological effects of individual furans, although 2,3,7,8-tetrachlorofuran was found to be highly toxic to chicks and guinea pigs (Moore et al., 1975).

This paper reports the detection of residues and determination of dioxins and furans in commercial gelatin. Gelatin is prepared by partial hydrolysis of collagen derived from skin, connective tissue, and bones of animals. Two types are produced commercially: type A by acid hydrolysis of porkskins and type B by alkaline hydrolysis of cattle bones and hides (Gelatin Manufacturers Institute of America, Inc., 1973). Both types are used in the food and pharmaceutical industries in the United States. Current annual consumption of gelatin in this country is estimated at 57 million pounds of domestic production and 13 million pounds of imports (Lewis, 1975). Contamination of cattle hides with PCP and dioxins is likely since PCP is used as a preservative during hide processing both in this country and abroad (Firestone, 1973; Lewis, 1975). The first indication that PCP may be a contaminant of gelatin came from analysis of a sample of unflavored gelatin purchased in Texas in April, 1975, as part of the Food and Drug Administration's (FDA's) Total Diet Studies. This finding led to examination of several commercially available gelatin samples for the presence of dioxins. A modification of the method of Baughman and Meselson (1973) was used for sample cleanup, and gelatin samples were detected and quantitated by EC–GLC. The presence of octachlorodioxin (OCDD) in several samples was confirmed by photodechlorination at 253.7 nm. Low levels (<1 ppb) of hepta- and octachlorofuran were also detected in some of the gelatins by EC–GLC. However, furans other than 2,3,7,8-tetrachlorofuran and octachlorofuran were not available for use as analytical standards. The presence of hexachlorodioxin (HCDD), heptachlorodioxin (HpCDD), and OCDD as well as several furans (penta-, hexa-, and heptachlorofuran) in one of the gelatin samples was confirmed with GLC–mass spectrometry.

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EXPERIMENTAL SECTION

Samples. Consumer samples of gelatin were purchased at a Washington, D.C. supermarket. Bulk samples and commercial gelatin blends were obtained by the Food and Drug Administration's Kansas City Laboratory.

Reagents. All solvents except ethanol were of "pesticide grade" and used as received. The ethanol (absolute, USP) and water were distilled in an all-glass apparatus. Florisil PR Grade, 60–100 mesh, activated by the manufacturer at 1250 °F (676 °C), was stored in the dark in a glass container and used as received.

Stock solutions of individual dioxins or furans were prepared by dissolving several milligrams of the reference chemical in 25 or 50 mL of isooctane. Aliquots of these solutions were added to isooctane to prepare standard solutions of 0.1 to 1.0 ng/μL. In addition, mixtures of hexa-, hepta-, and octachlorodioxins (0.5–1.0 ng/μL of each dioxin) were prepared from stock solutions. Purity of the reference chemicals (flame ionization and/or EC–GLC analysis) by GLC was as follows: TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), 99%; TCDF (2,3,7,8-tetrachlorodibenzofuran), 98%; 1,2,4,6,7,9-HCDD, 91%; 1,2,3,6,7,9-HCDD, 88%; 1,2,3,6,7,8-HCDD, 98%; 1,2,3,7,8,9-HCDD, 99%; 1,2,3,4,6,7,9-HpCDD, 91%; 1,2,3,4,6,7,8-HpCDD, 97%; OCDF (octachlorodibenzo-

furan), 98%; and OCDD, 99%. The HCDDs and HpCDDs were prepared by IIT Research Institute, Chicago, Ill. under FDA contract.

Florisil Chromatographic Columns. Disposable glass Pasteur pipets, total length 5.75 in. and body o.d. 7 mm, were used for preparation of chromatographic columns. Glass wool used for preparation of the chromatographic columns was first washed thoroughly with acetone and hexane (in a chromatographic column) and then air-dried and stored in a closed jar. A glass wool plug was placed in the bottom of each pipet and 4 cm of Florisil was added. The packed pipets, covered loosely with an "umbrella" of aluminum foil, were placed in an oven at 130 °C and held overnight before use. (The packed pipets may be kept for up to 4 weeks before use.) The packed pipets were removed from the oven and allowed to cool to room temperature (5 to 7 min) before elution of sample extract.

Apparatus. A Hewlett-Packard Model 5713A gas chromatograph equipped with a Model 18713A ^{63}Ni linear electron-capture detector was used for the analyses. A tube containing activated coconut charcoal was attached to the exit line from the detector to prevent air-borne dioxin or furan contamination of the laboratory. A Finnigan 3300F gas chromatograph/mass spectrometer interfaced to a Finnigan 6100 data system was used for confirmation of dioxins and furans in sample extracts. A 1 m × 0.2 cm i.d. GLC column was used, packed with 3% OV-101 on 80–100 mesh Chromosorb WHP, and programmed at 4 °C/min from 170 to 240 °C for the analyses. The photochemical reactor used for confirmation of OCDD by photodechlorination was a Rayonnet Model RPR 100, N.E. Ultraviolet Co., Middletown, Conn., fitted with light short-wavelength (253.7 nm) lamps. The lamps were covered with black tape, except for a 2-mm "slit" at the middle of each lamp in order to reduce the total light energy available to sample solutions. An Eberbach Model 6000 shaker was used to dissolve samples in alcoholic potassium hydroxide solution. All glassware was soaked in sulfuric acid–dichromate cleaning solution and rinsed thoroughly with distilled water and then glass-distilled acetone.

Extraction and Clean-Up Procedure. A reagent blank determination was carried out with each set of three samples. A 15-g sample of gelatin was weighed into a 100-mL round bottom flask. Twenty milliliters of ethanol and 40 mL of 40% aqueous potassium hydroxide were added, and the sample was dissolved by shaking 1.5–2 h in the shaker at about 180 strokes/min. The solution was transferred to a 125-mL separatory funnel, the flask rinsed with 10 mL of ethanol, and the rinsing added to the separatory funnel. The aqueous solution was extracted first with 20 mL of hexane and then with three additional 16 to 18-mL portions of hexane, and the hexane extracts were collected in the original 100-mL flask.

The combined hexane extracts were transferred to the original separatory funnel and extracted first with 20 mL of 1 N potassium hydroxide and then with 40-mL portions of concentrated sulfuric acid until the acid phase was colorless. The initial sulfuric acid extraction was carried out by swirling the separatory funnel, since vigorous shaking during the first extraction produced heavy emulsions. Moderate emulsions were broken up with a few crystals of anhydrous sodium carbonate.

The hexane extract was washed with 10 mL of water and 10 mL of saturated sodium carbonate solution and filtered through 5 cm (15 g) of anhydrous sodium carbonate in a 19 mm i.d. × 30 cm chromatographic tube. The hexane was collected in a clean 100-mL round-bottom (⅓ 14/20) flask. Three 20-mesh carborundum boiling chips were added to the flask, the flask was fitted with a modified micro-Snyder column (Kontes K 569251, size 3-14), and the hexane extract was concentrated to about 3 mL on the steam bath.

The concentrated hexane extract was passed through a column of Florisil in a Pasteur pipet prepared as described above. The column was eluted with 12 mL of hexane (eluate I) and then with 5 mL of methylene chloride (eluate II). Eluate II was collected in a 4-dram vial, and eluate I was discarded. Four milliliters of hexane was added to eluate II, which was then concentrated for EC–GLC analysis. A carborundum chip was placed in a concentrator tube (Kontes K-570050, size 124) and 1 mL of eluate II was added using a 9-in. Pasteur pipet to transfer the methylene chloride–hexane solution. The concentrator tube was fitted with a modified micro-Snyder column and the tip of the tube was placed in a steam bath to evaporate the solvent. The remainder of eluate II was added in 1-mL portions through the top of the micro-Snyder column, the 4-dram vial was rinsed with three 1-mL portions of hexane, and the rinsings were transferred through the top of the micro-Snyder column. Evaporation of solvent in the steam bath was continued to near dryness (about 5 μL) and the micro-Snyder column was removed. About 0.2 mL of hexane was added to the concentrator tube, and the solvent was again evaporated to near dryness. Addition and evaporation of hexane were repeated three more times. The tube was removed from the steam bath, hexane was added with a microliter syringe to give a final volume of 100 μL, and the tube was stoppered, mixed, and held for EC–GLC analysis.

EC–GLC Procedure. A 5-μL portion of Florisil eluate II (dissolved in 100 μL of hexane) was injected into the gas chromatograph operated as follows: glass column, 2 m long × 0.4 cm i.d., packed with 1.2% Silar 10C (cyanopropyl silicone) on Chromosorb WHP, 80–100 mesh; carrier gas, 5% methane in argon, 40 mL/min; recorder chart speed, 0.25 in./min; on-column injection; column, injector, and detector temperatures, 185–200, 250, and 300 °C, respectively. GLC analyses of hexa-, hepta-, and octachlorodioxins and OCDF were performed at a column temperature of 200 °C. The presence or absence of TCDD was determined by additional analysis at a column temperature of 185 °C. With TCDD eluting in 7–8 min (column temperature, 185 °C), 200 pg of TCDD caused 50% full-scale deflection or greater at 4× attenuation. Hexachlorodioxins eluted in 12–16 min at 200 °C. GLC peaks were identified on the basis of their retention times compared to the standards. A response vs. weight curve was prepared daily for quantitation of the sample peaks. The standard curve (peak height vs. nanograms of dioxin) was prepared from responses of standard solutions injected before and after injection of sample solutions during the course of the day.

Confirmation of OCDD by Photodechlorination. One-quarter to one-half of Florisil eluate II was transferred to a 10-mm i.d. × 140-mm glass-stoppered quartz tube and diluted with hexane to 0.5 mL. The tube was placed in the photochemical reactor for up to 30 min, and 5-μL portions were withdrawn at 2.5, 5, or 10 min intervals for EC–GLC analysis. A reference solution (0.5 mL of hexane) containing 1,2,3,4,6,7,9-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD was prepared to approximate the concentration of HpCDDs and OCDD present in the sample solution. The reference solution was irradiated simultaneously with the sample solution. OCDD in the sample solution was confirmed by comparison of the gas chromatograms of sample and reference solutions after various intervals of

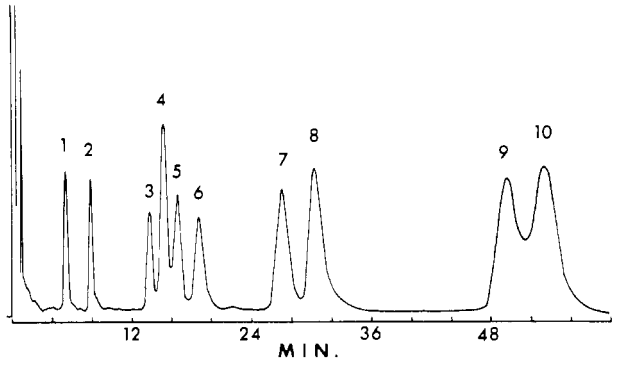

Figure 1. Gas chromatogram of a mixture of dioxins and furans: (1) 2,3,7,8-TCDD; (2) 2,3,7,8-TCDF; (3) 1,2,4,6,7,9-HCDD; (4) 1,2,3,6,7,9-HCDD; (5) 1,2,3,6,7,8-HCDD; (6) 1,2,3,7,8,9-HCDD; (7) 1,2,3,4,6,7,9-HpCDD; (8) 1,2,3,4,6,7,8-HpCDD; (9) OCDF; and (10) OCDD. (See text for GLC conditions and identity of dioxins and furans.)

irradiation. Qualitative observation of loss of OCDD and formation of HpCDDs with higher yields of 1,2,3,4,6,7,9-HpCDD relative to 1,2,3,4,6,7,8-HpCDD were considered sufficient for confirmation of OCDD. In addition, the following parameters were evaluated to determine their usefulness as confirmatory indices: (a) half-life ($t_{0.5}$) of OCDD (time required to reduce OCDD to one-half of its original concentration), (b) ratio of the HpCDD isomers produced by photodechlorination of OCDD, and (c) ratio of 1,2,3,4,6,7,9-HpCDD relative to OCDD at various irradiation times. The half-life of OCDD was determined from a plot of logarithm of peak height (mm) vs. irradiation time (min).

RESULTS AND DISCUSSION

Recovery Experiments. Initial evaluations of the Baughman–Meselson cleanup indicated that Florisil was superior to alumina for column chromatographic cleanup because Florisil gave better separations of dioxins and furans from potentially interfering compounds such as polychlorinated biphenyls (PCBs). A mixture of 800 ng of PCBs (Aroclor 1254, Monsanto, St. Louis, Mo.), 500 ng of *p,p'*-DDE and 15 ng each of TCDD and 1,2,4,6,7,9-HCDD was chromatographed on a column of Florisil (see Experimental Section). Greater than 99.5% of the PCBs and *p,p'*-DDE eluted in eluate I (hexane), whereas 100% of the dioxins and only a trace of the PCBs and *p,p'*-DDE were recovered in eluate II. A similar mixture was chromatographed on a column of neutral alumina. Eluate I (20% carbon tetrachloride in hexane) contained all of the added *p,p'*-DDE but only 94% of the PCBs. Eluate II contained all of the added dioxins and 6% of the added PCBs. Preliminary evaluations also demonstrated that a properly activated column adsorbent could be obtained most conveniently by prepacking the chromatographic tubes and holding them in the oven until ready for use.

The efficiency of the overall clean-up procedure for gelatin was tested with known amounts (1 to 3 ppb each) of ten dioxins and furans added to 15 g of control gelatin. A gas chromatogram of the mixture of dioxins and furans is shown in Figure 1. Silar 10C stationary phase was chosen for EC–GLC because it provided excellent resolution of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD (peaks 5 and 6). These components were poorly resolved with methyl silicone and methylphenyl silicone stationary phases. The identity and retention times of the dioxins and furans added to the gelatin are shown in Table I. Recoveries of the dioxins and furans from the samples fortified with 1 to 3 ppb of each compound are reported in Table II. Analyses were performed on three separate days, and two

Table I. Relative Retention Times (RRT)[a] of Dioxins and Furans

GLC peak no.	Compound[b]	RRT
1	2,3,7,8-TCDD	1.00
2	2,3,7,8-TCDF	1.50
3	1,2,4,6,7,9-HCDD	2.62
4	1,2,3,6,7,9-HCDD	2.89
5	1,2,3,6,7,8-HCDD	3.15
6	1,2,3,7,8,9-HCDD	3.60
7	1,2,3,4,6,7,9-HpCDD	5.16
8	1,2,3,4,6,7,8-HpCDD	5.83
9	OCDF	9.52
10	OCDD	10.2

[a] RRT = retention time relative to TCDD (glass coil, 2 m × 0.4 cm i.d., packed with 1.2% Silar 10C on 80–100 mesh Chromosorb WHP; column temperature, 200 °C. See text for additional GLC conditions. [b] TCDD = tetrachlorodioxin; TCDF = tetrachlorofuran; HCDD = hexachlorodioxin; HpCDD = heptachlorodioxin; OCDF = octachlorofuran; and OCDD = octachlorodioxin.

Table II. Recovery of Dioxins and Furans from Fortified Gelatin Samples[a]

Compound[b]	% recovery[c]		Coeff of variation[c]
	Range	Av	
1	87–100	94	6.5
2	83–93	89	5.3
3	94–109	103	6.8
4	90–103	99	7.4
5	88–102	97	8.0
6	85–103	94	5.8
7	84–95	91	6.4
8	86–98	93	3.9
9	36–40	39	7.4
10	64–78	70	6.8

[a] One-three parts per billion of each compound in 15 g of gelatin. [b] See Table I for identity of compounds. [c] Six determinations.

spiked samples and a sample blank were analyzed on each day. Recoveries were: TCDD, 87–100%; HCDDs, 85–109%; HpCCDs, 84–97%; OCDD, 64–78%; TCDF, 83–93%; and OCDF, 36–40%.

The moderate recoveries of OCDD and poor recoveries of OCDF prompted investigation of the individual steps involved in sample cleanup. Recoveries were determined for 50-ng portions of OCDF or OCDD subjected to (1) sulfuric acid extraction, (2) sulfuric acid extraction and Florisil chromatography, (3) the entire cleanup (no gelatin), and (4) the entire cleanup with 15 g of gelatin. Recoveries of 98% OCDF and OCDD were obtained after sulfuric acid extraction only. Recoveries were lowered to 90% (OCDF) and 80% (OCDD) after sulfuric acid extraction, followed by Florisil column chromatography. Approximately 70% recoveries were obtained when OCDF or OCDD was subjected to the entire cleanup without gelatin sample. However, recovery of OCDF was reduced to 40% when the entire cleanup was carried out in the presence of gelatin. The results indicated that OCDF was lost primarily during the alkali dissolution step (presumably due to hydrolysis) and that losses of OCDD occurred during Florisil column chromatography as well as during the alkali dissolution step.

EC–GLC Analysis of Gelatin Samples. A series of domestic and imported gelatin samples was examined; their identities and PCP contents are shown in Table III. PCP was determined by extraction with petroleum ether–ethyl ether (1:3) in the presence of dilute sulfuric acid (high-speed blending), extraction into alkali solution, acidification and reextraction into ethyl ether, methylation

Table III. Identity of Gelatin Samples Analyzed for Dioxin Content

Sample no.	Identity	% Mexican gelatin A in blend[a]	PCP,[b] ppm
1	Bulk domestic pork skin gelatin		0.0
2	Bulk domestic pork skin gelatin		0.0
3	FDA Total Diet (1975) sample A (consumer package)		3.8
4	FDA Total Diet (1975) sample B (consumer package)		6.4
5	Consumer package, unflavored[c]		
6	Consumer package, unflavored[c]		
7	Consumer package, unflavored[c]		
8	Bulk gelatin imported from Colombia, South America		0.01
9	Bulk gelatin imported from Mexico, manufacturer A	100	3.5
10	Bulk gelatin imported from Mexico, manufacturer A	100	7.5
11	Bulk gelatin imported from Mexico, manufacturer A	100	8.3
12	Bulk gelatin imported from Mexico, manufacturer B	0	0.3
13	Commercial blend, domestic pork skin gelatin and Mex. A	33	2.2
14	Commercial blend, domestic pork skin gelatin and Mex. A	35	3.1
15	Commercial blend, domestic pork skin gelatin and Mex. A	9	1.0

[a] Gelatin imported from Mexico, manufacturer A (Mex. A). [b] PCP = pentachlorophenol. Analysis by Kansas City Laboratory, FDA. Analysis of sample 8 by FDA, Washington, D.C. Current methodology for PCP in gelatin may be subject to considerable quantitative variation. [c] Purchased in Washington, D.C. supermarket; not analyzed for PCP.

Table IV. Dioxin Content of Gelatin Samples[a]

	Dioxins,[b] ppb							
Sample no.	1,2,4,6,7,9-HCDD	1,3,6,7,9-HCDD	1,2,3,6,7,8-HCDD	1,2,3,7,8,9-HCDD	1,2,3,4,6,7,9-HpCDD	1,2,3,-4,6,7,8-HpCDD	OCDD	Total dioxins
1	0.00	0.00	0.00	0.00	0.01	0.0	0.1	0.1
2	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0
3[c]	0.00	0.2	0.00	0.03	0.0	0.1	0.2	0.6
4[c]	0.00	0.2	0.00	0.04	0.0	0.3	0.4	1.0
5[c]	0.00	0.00	0.00	0.00	0.02	0.02	0.1	0.2
6[c]	0.03	0.2	0.03	0.05	0.2	0.16	0.2	0.8
7	0.1	0.7	0.4	0.09	0.8	0.8	0.6	3.6
8	0.00	0.00	0.00	0.00	0.2	0.2	0.6	0.9
9[c]	0.02, 0.03	0.3, 0.3	0.4, 0.6	0.05, 0.02	3.8, 3.9	4.6, 5.3	20, 16	30, 26
10[c]	0.02, 0.02	0.1, 0.1	0.3, 0.2	0.05, 0.09	2.5, 2.7	2.8, 2.9	20, 17	25, 23
11[c]	0.02, 0.02	0.2, 0.4	0.6, 0.8	0.07, 0.2	3.5, 4.0	3.6, 5.0	21, 18	29, 28
12	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.02, 0.02	0.02, 0.02	0.1, 0.1	0.1, 0.1
13[c]	0.01, 0.01	0.06, 0.08	0.2, 0.3	0.02, 0.09	0.9, 0.9	1.2, 1.2	4.8, 4.3	7.0, 6.9
14[c]	0.01, 0.01	0.05, 0.08	0.1, 0.2	0.02, 0.07	0.6, 0.5	0.6, 0.8	2.9, 1.9	3.8, 3.6
15[c]	0.01, 0.01	0.02, 0.03	0.04, 0.09	0.01, 0.02	0.2, 0.3	0.3, 0.4	1.4, 1.1	2.0, 2.0

[a] Dioxins identified on basis of GLC retention times compared to standards. [b] HCDD = hexachlorodioxin; HpCDD = heptachlorodioxin; OCDD = octachlorodioxin. (See text for limits of detection of the dioxins.) [c] Heptachlorofuran (ca. 0.1–0.4 ppb) and octachlorofuran (ca. 0.05–0.4 ppb) detected in sample.

with diazomethane, and determination by EC–GLC with a microcoulometric detector (Trotter, 1975). The PCP method is subject to considerable quantitative variation. Fifteen samples, including both commercial bulk samples (imported and domestic) and consumer packages, were analyzed for dioxins and furans, with the results shown in Table IV. Results for dioxins were not corrected for recoveries. Results for OCDF were corrected, based on a recovery value of 40%. Limits of quantitation (about 2.5% of full-scale deflection at 8× attenuation) were about 0.006, 0.012, and 0.018 ppb (5, 9, and 14 pg) for the hexa-, hepta-, and octachlorodioxins, respectively ($^1/_{20}$ of the extract solution from a 15-g sample was chromatographed). The limit of quantitation for OCDF was about 0.02 ppb. Single analyses were reported for samples 1–8. Duplicate analyses reported for samples 9–15 represent single analyses performed on separate days. Figure 2 is a gas chromatogram (Florisil eluate II) of sample 11 (bulk gelatin, Mexican manufacturer A). Reagent blanks were negative, yielding chromatograms that indicated no interference with analysis of hexa-, hepta-, and octachlorodioxins and OCDF.

Samples 9–11, three lots of gelatin imported from Mexico (manufacturer A), contained the highest levels of dioxins, of which 64% or more was OCDD. Samples 7 and 13–15 contained the next highest levels. The other samples contained low levels. Dioxin levels in the gelatin samples were roughly proportional to the PCP levels in the samples (about 1:300 for samples 10, 11, 12, 13, and 15). Samples that contained only a trace or no detectable PCP were found to contain only a trace or no dioxins. Heptachlorofuran (HpCDF) and OCDF were detected at levels of 0.05 to 0.4 ppb in ten of the samples (Table IV). The furans were identified by comparison of EC–GLC retention times in sample extracts with retention times of compo-

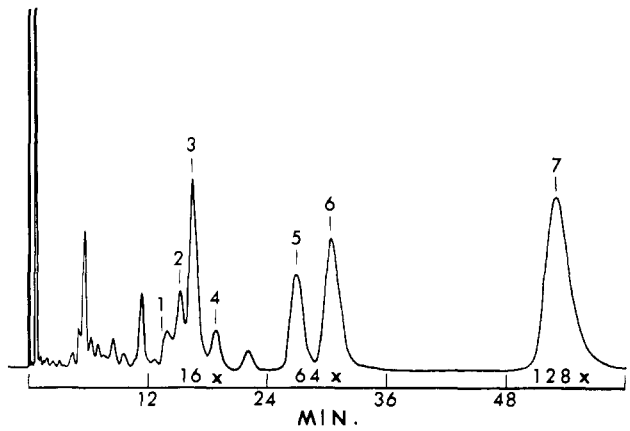

Figure 2. Gas chromatogram of extract (Florisil eluate II) from 750 mg of gelatin (sample 11) imported from Mexico: (1) 1,2,4,6,7,9-HCDD; (2) 1,2,3,6,7,9-HCDD; (3) 1,2,3,6,7,8-HCDD; (4) 1,2,3,7,8,9-HCDD; (5) 1,2,3,4,6,7,9-HpCDD; (6) 1,2,3,4,6,7,8-HpCDD; and (7) OCDD. (See text for GLC conditions.)

nents in the OCDF standard, which contained minor amounts of three HpCDFs as demonstrated by GLC–MS analysis (Pomerantz, 1975). OCDF levels in the samples were estimated by using an OCDF standard solution and correcting for losses (60%) during sample cleanup. HpCDF levels were estimated on the basis of HpCDD response.

The presence of dioxins and furans (and PCP) in the consumer (market) samples is suspected to be due to blending of PCP-contaminated imported gelatin with domestic gelatin by the manufacturer. Investigations by the FDA have shown that dioxins are readily formed by heating and pyrolysis of commercial chlorophenols (Higginbotham et al., 1968) and that hexa-, hepta-, and octachlorodioxins as well as tetra-, penta-, hexa-, hepta-, and octachlorofurans are present in commercial PCPs (Firestone et al., 1972). No TCDD was found in any of the samples (limit of detection about 0.2 to 0.4 ppb).

Confirmation of OCDD by Photodechlorination. Earlier work (Kim et al., 1975) demonstrated that dioxins undergo photochemical dechlorination in methanol or hydrocarbon solvents under UV irradiation. First-order kinetic rate data are obtained. The mechanism of photodechlorination involves breakage of the C–Cl bond and hydrogen atom addition. The initial products formed are isomers of $(n - 1)$ chlorodioxins, where n is the number of chlorine atoms on the dioxin ring prior to UV irradiation. The number and position of the chlorine atoms on the dioxins ring affect dechlorination in a predictable manner so that the decomposition rate and composition of the dechlorination products can be used to identify the starting dioxin.

Figures 3 and 4 (semilog plots) show the rates of photodechlorination of OCDF and OCDD, respectively. The half-lives ($t_{0.5}$) under the conditions employed here (0.5 mL of hexane solution containing less than 0.5 ng of HpCDD or OCDD/μL, irradiated as described in the Experimental Section) were 2.5 min (OCDF) and 5.0 min (OCDD). Investigation of the photodechlorination rate and ratio of products obtained from irradiation of OCDF or OCDD indicated that these would be useful for confirming the presence of OCDF or OCDD in the gelatin samples. It did not appear feasible to use this technique to confirm the other dioxins because of the complex mixture of products obtained and/or the lower levels of the other dioxins present in the samples. Irradiation of OCDF provided a useful technique for preparation of otherwise unavailable

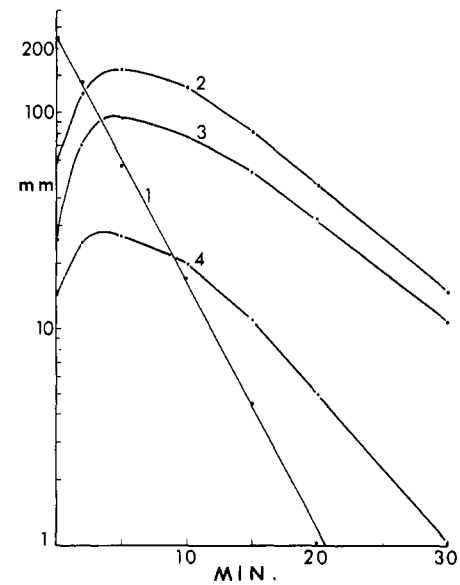

Figure 3. Photodechlorination of octachlorodibenzofuran (OCDF); 200 ng/500 μL of hexane solution. GLC peak height (mm) vs. irradiation time (min) at 253.7 nm. (1) OCDF; (2), (3), (4) heptachlorodibenzofurans with retention times relative to OCDF of 0.46, 0.52, and 0.67, respectively. EC–GLC column temperature, 210 °C.

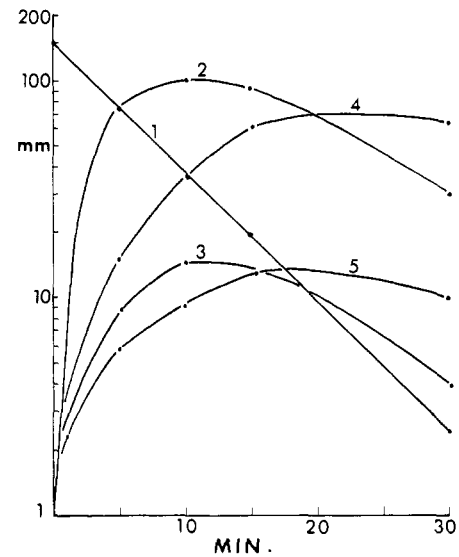

Figure 4. Photodechlorination of octachlorodibenzo-*p*-dioxin (OCDD); 200 ng/500 μL of hexane solution. GLC peak height (mm) vs. irradiation time (min) at 253.7 nm. (1) OCDD; (2) 1,2,3,4,6,7,9-HpCDD; (3) 1,2,3,4,6,7,8-HpCDD; (4) 1,2,4,6,7,9-HCDD; and (5) 1,2,3,6,7,9-HCDD. EC–GLC column temperature, 210 °C. (See Table I for additional GLC conditions.)

HpCDFs in an HpCDF–OCDF mixture for use as a qualitative standard for detection of these components in samples (Buser, 1976).

Six of the gelatin samples (samples 9–11 and 13–15) with OCDD levels of 1–20 ppb were selected to explore the utility of photodechlorination for OCDD confirmation. The low levels of OCDF detected in the gelatins by GLC (Table IV, footnote *c*) precluded use of photodechlorination to confirm its presence in the samples. Since commercial PCPs contain approximately equal amounts of OCDF and OCDD (Buser and Bosshardt, 1976), it appears that the levels of OCDF originally present in the PCP-contaminated cattle hides were substantially reduced by various storage and processing factors including alkaline hydrolysis of the hides during gelatin manufacture.

Table V. Photoirradiation of Gelatin Extracts at 254 nm in Hexane Solution[a]

	Irradiation time, min																
	0		2.5		5		10		15		20		25		30		$t_{0.5}$ OCDD,
Sample	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	min
9	0.9	0.3	1.8	0.8	2.3	1.3	2.9	2.5	3.4	4.5	3.5	6.9			4.2	13.8	5.6
10	1.0	0.2	2.1	0.6	2.9	1.1	3.7	2.3	4.0	4.0	4.2	5.9			5.9	13.1	5.4
11	1.0	0.3	2.0	0.7	2.6	1.2	3.4	2.6	3.7	4.3	3.9	7.4			5.4	19.0	5.6
Mixture 1	0.9	0.3	1.6	0.9	2.1	1.5	2.6	3.3	3.3	5.9	3.4	9.8			4.3	15.0	5.2
13	1.0	0.4			1.9	1.1	2.5	2.1	2.9	3.1			3.5	7.5			8.5
14	1.0	0.5			2.2	1.6	2.7	2.8	2.9	4.5			3.7	9.3			6.5
15	0.9	0.4			2.1	1.5	2.6	2.6	2.8	3.3			3.6	12.5			6.5
Mixture 2	1.1	0.4			2.3	1.9	2.7	3.2	3.2	5.4			3.7	11.3			5.5

[a] Mixtures 1 and 2 were prepared with heptachlorodibenzo-p-dioxin (HpCDD) and octachlorodibenzo-p-dioxin (OCDD) standards to yield hexane solutions with HpCDD and OCDD composition similar to that of the samples. R_1 = ratio of (EC-GLC) peak height of 1,2,3,4,6,7,9-HpCDD to that of 1,2,3,4,6,7,8-HpCDD; R_2 = ratio of peak height of 1,2,3,4,6,7,9-HpCDD to that of OCDD. $t_{0.5}$ OCDD = half-life (minutes) of OCDD determined from a semilog plot of OCDD concentration vs. irradiation time.

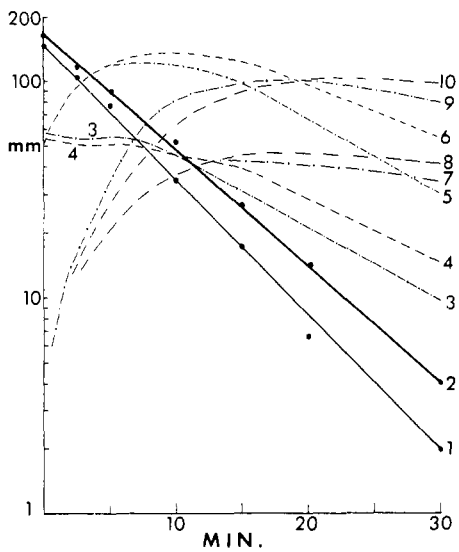

Figure 5. Photodechlorination of reference mixture (hexa- and heptachlorodioxins) and sample 9 extract (Florisil eluate II). GLC peak height (mm) vs. irradiation time (min) at 253.7 nm. (1) reference OCDD; (2) sample OCDD; (3) reference 1,2,3,4,6,7,8-HpCDD; (4) sample 1,2,3,4,6,7,8-HpCDD; (5) reference 1,2,3,4,6,7,9-HpCDD; (6) sample 1,2,3,4,6,7,9-HpCDD; (7) reference 1,2,3,6,7,9-HCDD; (8) sample 1,2,3,6,7,9-HCDD; (9) reference 1,2,4,6,7,9-HCDD; and (10) sample 1,2,4,6,7,9-HCDD. EC–GLC column temperature, 210 °C. (See text for additional GLC conditions.)

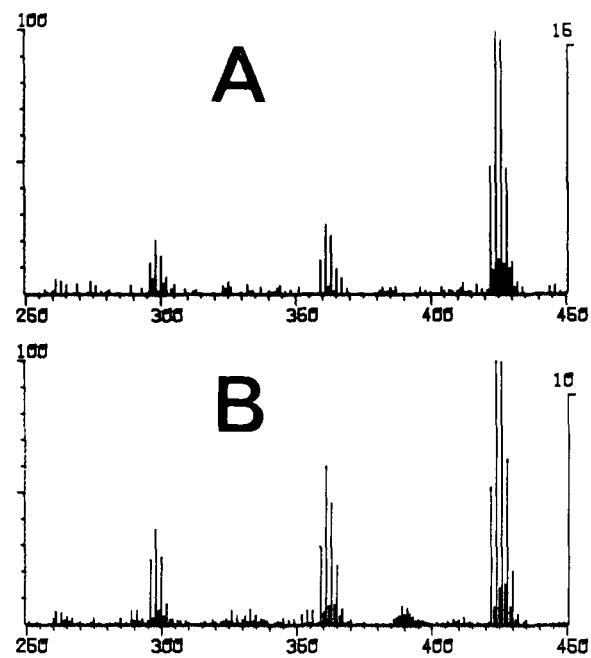

Figure 6. Mass spectra of (A) 1,2,3,4,6,7,8-HpCDD standard and (B) HpCDD in extract from gelatin (sample 9) imported from Mexico.

One-quarter or one-half of each of the sample extracts (Florisil eluate II), diluted to provide 0.5 mL of hexane solution, was irradiated for about 30 min, and 5-μL portions were withdrawn at 2.5 to 10-min intervals for EC–GLC analysis. Hexane solutions of mixtures of HpCDDs and OCDD were prepared to approximate the concentration of these components in the sample solutions, and the reference mixtures were irradiated simultaneously with the samples. The presence of OCDD was confirmed by observation of the sample decomposition pattern (loss of OCDD and formation of HpCDDs), as well as by the approximate $t_{0.5}$ of OCDD and ratio of HpCDDs produced as a result of primary photodechlorination of the OCDD. Figure 5 shows rate curves for decomposition of OCDD (sample 9) and for appearance and decomposition of the HpCDD and HCDD irradiation products. Nearly parallel curves were obtained for the sample and the reference mixture. The photochemical data obtained from irradiation of the six samples are shown in Table V. $t_{0.5}$ values for sample OCDD were slightly higher than those for the reference mixtures, probably because of sample matrix effects. Ratios of 1,2,3,4,6,7,9-HpCDD to 1,2,3,4,6,7,8-HpCDD (R_1) and ratios of 1,2,3,4,6,7,9-HpCDD to OCDD (R_2) in the sample solutions were in rough agreement with those in the reference mixtures.

Confirmation of Dioxins and Furans by GLC–MS. The presence of dioxins and furans in gelatin sample 9 (bulk gelatin, Mexican manufacturer A) was confirmed by GLC–MS. A 75-g portion of sample was dissolved in alcoholic potassium hydroxide solution (60 mL of ethanol and 120 mL of 40% aqueous potassium hydroxide) by shaking 2 h at room temperature, and the sample was extracted with three 60-mL portions of hexane. The hexane solution was then washed with concentrated sulfuric acid, filtered through anhydrous sodium carbonate, and chromatographed on Florisil as described in the Experimental Section. The extract was examined by GLC–MS and dioxins and furans were identified on the basis of characteristic mass spectral fragmentation patterns (Firestone et al., 1972). HCDD, HpCDD, and OCDD standards, as well as a mixture of 2,4,6,8-tetra- and

1,2,4,7,8-pentachlorofurans, were examined, along with the sample extract. A HCDD standard was used to determine instrument sensitivity at the time of the analyses. It was estimated that dioxins present at a level of 0.5 ppb or higher would have been identified on the assumption that all dioxins in the sample gave a similar response in the mass spectrometer. The presence of the following components was confirmed in the sample: HCDDs, HpCDDs, OCDD, and penta-, hexa-, and heptachlorofuran. Results obtained in the mass spectral analysis are illustrated in Figure 6, which shows the mass spectrum of a 1,2,3,4,6,7,8-HpCDD standard (A) as well as that of the HpCDD component in the sample (B) with the same GLC retention time as the standard. The typical M – (CO + Cl) and M – 2(CO + Cl) fragments are seen in both standard and sample spectra, as well as the HpCDD molecular ion cluster.

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Individual Lipids and Proximate Analysis of Various Foods. 1. French Fried Potatoes from Ten Chain Restaurants

Willard D. Hubbard,* Allan R. Prosser, Alan J. Sheppard, David R. Newkirk, Theodore Osgood, Elias Tombropoulos, and Shirley T. Jones

French fried potatoes were obtained every third day from ten chain restaurants for a total of six samplings from each restaurant. The samples were analyzed for water, total fat, fatty acids, protein, ash, sterols, and *cis,cis*-methylene interrupted polyunsaturated triglycerides. The data show that variations exist in the day-to-day selection of cooking oils used within a single restaurant. Also, the wide variations in data from one restaurant to another indicate that different oils were being selected. Values for total fat ranged from 14.0 to 28.4 g/100 g of product, and cholesterol values varied from near zero to 19.0 mg/100 g of product. Several restaurants appeared to use mixtures of animal and vegetable fats. One establishment used only a vegetable oil and others used only animal fat.

More information is needed on the analysis of foods supplied by fast-food chain restaurants in view of the relatively large number of meals consumed by the public in these establishments. Of particular interest is the cholesterol and fatty acid content of foods. Samples of french fried potatoes were obtained from ten major fast-food chain restaurants. Water, total fat, fatty acids, protein, ash, sterols, and *cis,cis*-methylene interrupted polyunsaturated triglycerides were determined.

MATERIALS AND METHODS

Ten restaurants were each visited a total of six times in a sequence of every third day for collection of samples. The restaurants were Arby's, Burger Chef, Burger King, Gino's, Hardee's, Hot Shoppe Jr., Jack in the Box, McDonald's, Red Barn, and Roy Rogers. (The data in the tables do not reflect this order of listing.) Samples were homogenized in a Waring blender and stored at −2 °C for a few days before extraction. The extraction procedure, using chloroform–methanol, has been described by Sheppard et al. (1974). A sufficient amount of sample was taken for the extraction step so that approximately 1 g of fat was recovered. The methyl esters of the fatty acids were prepared by the procedure of the Association of Official Analytical Chemists (AOAC, 1975) as modified by Solomon et al. (1974). The method for the preparation of the butyrate derivatives for sterol analysis and the method for cholesterol have been described by Sheppard et al. (1974). Official methods of the AOAC (1975) were used

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