Low-Resolution Multiple Ion Detection Gas Chromatographic–Mass Spectrometric Comparison of Six Extraction–Cleanup Methods for Determining 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Fish

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Six laboratories participated in a study of six extraction–cleanup procedures for determining 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD) in fish. Six samples (three unfortified and three fortified with TCDD) were sent to each laboratory for extraction–cleanup according to the procedure(s) in use by each laboratory at that time. Sample extracts were returned to the Bureau of Foods, Food and Drug Administration (BF/FDA), for examination by capillary gas chromatography–low-resolution multiple ion detection mass spectrometry (GC–MS), using 12 ions. In addition, electron capture GC and full-scan GC–MS were employed to give additional measures of cleanup efficiency. Under the GC–MS conditions used by BF/FDA to examine the sample extracts, the Dow and Fish and Wildlife Service procedures were most efficient. The ranking of the other procedures, in order of decreasing efficiency, was New York State Department of Health, FDA, Environmental Protection Agency (EPA) neutral, and EPA acid–base.

A number of analytical methods are used to determine low parts per trillion (ppt) levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in fish as well as other environmental samples (Lamparski et al., 1979; O'Keefe et al., 1978; Baughman and Meselson, 1973; DiDomenico et al., 1979; Huckins et al., 1978; Baughman, 1974; Fanelli et al., 1980; Fukuhara et al., 1975; Crummett and Stehl, 1973). These methods may be separated into two parts: the extraction–cleanup phase and the quantitation–confirmation phase. Currently, gas chromatography–mass spectrometry (GC–MS) is used to quantitate and confirm the identity of TCDD (Hass et al., 1978, 1979; Buser, 1977; Hummer and Shadoff, 1980; Nestrick et al., 1979; Harless and Oswald, 1978; Buser and Rappe, 1978; Shadoff and Hummel, 1978; Reynolds et al., 1977; Hunt et al., 1975). Also, GC with electron capture detection (GC–EC) may be used to screen extracts for the presence of TCDD before GC–MS analysis.

In view of the variety of methods in use, a comparison of the extraction–cleanup phase of the various methods would be useful to identify the most efficient cleanup procedures currently available. The relative efficiency of the cleanups is determined in the present work by the following two criteria: (1) the relative number and amounts of undesired components which were present in the final extracts resulting from the application of the extraction–cleanup procedure and (2) by the extent to which these components interfered with the TCDD peak by having similar GC retention times. The objective of this study was to compare the overall efficiency of available analytical cleanup procedures determined by GC–MS as developed in the Bureau of Foods, Food and Drug Administration (BF/FDA), for quantitating and confirming the identity of TCDD in fish. This procedure uses capillary column GC and 12-ion multiple ion detection (MID) low-resolution MS. As additional measures of the cleanup efficiency, sample extracts were screened and quantitated by GC–EC and finally analyzed by full-scan GC–MS.

Six laboratories participated in the study: BF/FDA, Detroit District (DET/FDA), Dow Chemical Co. (Dow), Environmental Protection Agency (EPA), Fish and Wildlife Service (FWS), and New York State Department of Health (NYS). Each laboratory received six fish samples, three of which were fortified with TCDD. These samples were extracted by analysts in the laboratories according to the procedures then in use by each of them and the purified extracts were sent to BF/FDA, Washington, DC, for analysis by GC–MS and GC–EC. For the purposes of this study, only Dow added an internal standard to the samples prior to initiating cleanup to facilitate quantitation by BF/FDA.

This study did not evaluate the overall analytical method used by any of the participating laboratories, including individual GC–MS procedures. The results of the FDA evaluations of cleanup efficiency do not necessarily reflect upon the validity of TCDD analyses performed by the participating laboratories using their combined extraction–cleanup–MS procedures.

EXPERIMENTAL SECTION

Sample Preparation. The samples were prepared from fish collected by FDA in Saginaw Bay, MI, in Nov 1978. The fish were filleted, ground, placed in clean quart jars, and frozen by DET/FDA until shipment in Jan 1979 to BF/FDA where the samples were held frozen until processed for this study. Twenty-gram portions of each sample (or 10-g portions for Dow Chemical Co.) were weighed into 2-oz wide-mouth jars. Fortified samples were prepared as follows. Half of the sample was added to the jar, covering the bottom of the jar with sample and forming a shallow, bowl-shaped depression in the middle of the sample. Next, 30 μL (20-g sample) or 15 μL of a standard TCDD solution (10-g sample) was added to the bowl-shaped depression by using a solution of 70 pg of TCDD/μL of ethanol–isooctane (3:2). The remaining half of the sample was then added to completely cover the first half of the sample. The fortified samples therefore contained 105 ppt of TCDD (except samples prepared for cleanup by BF/FDA which contained 121 ppt of TCDD). The jar was covered with acetone-washed aluminum foil, dull side down; the screw cap was secured with moderate pressure. The jar was labeled with the sample number and placed in the freezer with the unfortified samples.

Each set of samples consisted of the following: sample 1, sucker, sample 2, sucker fortified with 105 (121) ppt of TCDD; samples 3 and 5, catfish; samples 4 and 6, catfish fortified with 105 (121) ppt of TCDD. Samples 1 and 2 were prepared from a single homogenate of sucker, and

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samples 3–6 were prepared from a single homogenate of catfish. Reserve portions of the sucker and catfish homogenates were also prepared to provide participating laboratories with additional unfortified materials for practice or internal (in-house) method evaluation.

Sample Distribution. The samples were packed in dry ice and shpped to participating laboratories via fast airfreight with instructions to the analysts to conduct extraction and cleanup of the samples and reagent blanks according to their usual method and to return sample extracts and reagent blanks dissolved in 60–100 μL of hexane and sealed in 3 mm o.d. glass tubes. Participants were also instructed to provide a written description of their extraction–cleanup procedures.

Extraction–Cleanup Methods. A variety of extraction–cleanup procedures have been developed for analysis of fish and other biological samples for TCDD residues. Initial approaches to cleanup methodology were derived from the procedure of Baughman (1974) and Baughman and Meselson (1973), which involves a base and acid treatment of the sample, followed by two column chromatographic steps. More recent attempts to improve the cleanup and reduce or eliminate observed interferences in subsequent GC–MS analysis led to cleanup procedures that eliminated the use of acid or base, introduced the use of dual chromatographic columns, and incorporated high-performance liquid chromatographic (HPLC) cleanup steps.

The procedures usually used by the participating laboratories are briefly described below. In the current study, only the Dow samples were fortified with an internal standard before cleanup.

EPA Acid–Base Cleanup (EPA-A/B) (Harless et al., 1980). The sample (10–20 g) is fortified with a [^{37}Cl]TCDD internal standard, refluxed with KOH, and extracted with hexane. The hexane extracts are extracted with concentrated sulfuric acid, dried on a sodium carbonate column, and chromatographed on a neutral alumina column. Carbon tetrachloride, followed by methylene chloride, is used as the eluant.

EPA Neutral Cleanup (EPA-Neut) (Harless et al., 1980). The sample (15–20 g) is blended with anhydrous sodium sulfate and dry ice, and the resultant powder is fortified with a [^{37}Cl]TCDD internal standard and extracted with acetonitrile. A portion of the acetonitrile extract is extracted with acetonitrile-saturated hexane, and the hexane layer is discarded. The acetonitrile is concentrated to a small volume and replaced with hexane. The extract, in a small volume of hexane, is chromatographed first on a Florisil column and then on a neutral alumina column, in each case eluting sequentially with 100% hexane, 10% methylene chloride in hexane, and 25% methylene chloride in hexane.

FDA Acid–Base Cleanup (FDA) (Firestone, 1977; Firestone et al., 1979). The sample (20 g) is dissolved in alkaline solution by shaking 2–3 h at room temperature. The solution is extracted with hexane, and the hexane extracts are extracted with concentrated acid, dried on a sodium carbonate column, and chromatographed on a neutral alumina column, eluting with 20% carbon tetrachloride in hexane and then methylene chloride. The methylene chloride eluate is chromatographed on a Florisil column, eluting with 10% methylene chloride in hexane and then methylene chloride. The TCDD fraction (methylene chloride eluate) is finally subjected to HPLC (Zorbax-ODS column; 40 °C) with methanol as the eluant.

New York State Neutral Cleanup (NYS) (O'Keefe et al., 1978). The sample (1–20 g) is fortified with a [^{37}Cl]-TCDD internal standard, blended with methylene chloride and sodium sulfate, and filtered. The methylene chloride extract is evaporated, and the methylene chloride is replaced with hexane. The solution is loaded on a dry packed, magnesia–Celite 545 column, and eluted with ethyl ether–hexane, followed by benzene. The benzene extract is chromatrographed on a neutral alumina column, eluting with carbon tetrachloride and methylene chloride, and the methylene chloride eluate is chromatographed on a Florisil column, eluting with hexane and methylene chloride.

Fish and Wildlife Service Cleanup (FWS) (Huckins et al., 1978). The sample is blended with anhydrous sodium sulfate until a free-flowing powder is obtained. The mixture is packed into a glass column and extracted with methylene chloride (200 mL of methylene chloride for each 20 g of fish). The solution is evaporated to ~50 mL, and methanol and benzene are added to yield a solution containing 20% methanol and 5% benzene. The solution is passed through a carbon–glass fiber sorbent, and the sorbent is washed with methylene chloride–methanol–benzene (75:20:5). The TCDD is then eluted with toluene and chromatographed first on a composite column of potassium silicate or cesium silicate over sulfuric acid–silica gel, eluting with hexane, and then on an acid alumina column, eluting with hexane and methylene chloride–hexane.

Dow Chemical Co. Cleanup (Dow) (Lamparski et al., 1979). The sample (10–20 g) is fortified with a [^{13}C]TCDD internal standard and shaken 1 h with concentrated hydrochloric acid. The sample, which is completely dissolved, is then extracted with hexane (overnight shaking plus an additional 3-h shaking). The hexane extract is passed first through a dual-column system of silica, concentrated sulfuric acid on silica, and 1 M aqueous sodium hydroxide on silica, followed by a second dual-column system of silver nitrate on silica and basic alumina. The dioxin fractions are then cleaned up by normal phase silica (Zorbax-SIL; hexane solvent) HPLC, followed by reverse-phase (Zorbax-ODS; methanol solvent) HPLC.

Preparation of Sample Extracts. Sealed glass tubes containing the sample extracts (60–100 μL) were rinsed with distilled-in-glass acetone and placed in a steam bath for 20 s before removal of extract. After they were cooled to room temperature, the sample tubes were broken ~10 mm above the column of liquid and 3–5 μL was withdrawn for GC–EC screening. The remainder of the extracts was transferred to 1-mL concentrator tubes (Kontes K-570050; size 124; 14/20), rinsing the sample tubes with small portions of isooctane to give a final volume of ~1 mL. The stoppered concentrator tubes were held for GC–MS analysis.

Electron Capture Gas Chromatographic Screening. GC–EC screening was carried out using a 1.8 m × 4 mm i.d. glass GC column packed with 1.2% Silar-10C on 80–100-mesh Chromosorb W-HP. A Hewlett-Packard Model 5713 gas chromatograph was used with a ^{63}Ni linear EC detector and EC controller to maintain 10^{-9}-A standing current. Operating temperatures were as follows: column, 170 °C; injection port, 250 °C; detector, 300 °C. Carrier gas was 95% Ar–5% CH_4 with a flow rate of 40 mL/min. By use of these parameters, TCDD eluted in ~20 min, and 140 pg of TCDD caused ~50% full-scale deflection at 4× attenuation of the EC controller. TCDD was quantitated by injection of 5.0 μL of sample extract ($^1/_{20}$ of extract solution) and comparison of the height of the sample peak with retention time of TCDD to the peak height of a TCDD standard. After elution of TCDD from the column, the column temperature was programed at 32 °C/min to

200 °C and held for ~16 min to elute components with retention times longer than TCDD.

Multiple Ion Detection Gas Chromatographic–Mass Spectrometric Procedure. The GC–MS analyses involved use of a wall-coated open tubular glass capillary column about 10 m × 0.25 mm i.d. coated with OV-101 prepared in house and exhibiting routinely between 25 000 and 40 000 theoretical plates. The glass column was directly coupled via a transfer line to a Finnigan 3300 F mass spectrometer. An SGE SCI-A splitless capillary injector was used in the injection port of the Finnigan 9500 gas chromatograph. The mass spectrometer was operated under low-resolution electron impact conditions and was interfaced with a Finnigan INCOS 2300 data system. MS conditions were as follows: electron energy, 24 eV; filament emission, 0.50 mA; preamplifier, 10^{-8} A/V; multiplier, ~2200 V.

MID parameters were as follows: standard revision 3.1(C) software; 12 ions monitored at m/z 257, 259, 261, 305, 307, 320, 321, 322, 324, 326, 332, and 334; total scan time, 2.6 s. The 12 ions include 8 ions characteristic of TCDD. The molecular ion cluster consists of m/z 320, 322, 324, and 326. The ion at m/z 321 provided verification of the normal isotope abundance relative to m/z 320 and gave supporting evidence that responses at m/z 320, 322, 324, and 326 attributed to TCDD did not arise as an isotope response from ions at m/z 319, 321, 323, and 325 from another compound. The fragment ion cluster representing the loss of COCl· from the molecular ion cluster was monitored at m/z 257, 259, and 261. Ions at m/z 332 and 334 were monitored from the molecular ion cluster of the [$^{13}C_{12}$]TCDD internal standard. Ions at m/z 305 and 307 were monitored to detect a possible interference from tetrachloromethoxybiphenyl compounds fragmenting by loss of methyl, which could invalidate quantitation based on the molecular ion cluster of TCDD (Phillipson and Puma, 1981).

As internal standard (5 μL of 260 ng/μL fully labeled [^{13}C]TCDD supplied by the National Center for Toxicological Research, Jefferson, AR) was added to the sample extract as received (except Dow sample extracts, which contained an internal standard). The extract and washings were transferred to a calibrated Kontes 2.0-mL Chromaflex tube and concentrated to ~10 μL. Normally, 1 μL was injected in the splitless mode and the chromatograph temperature programmed from 65 to 240 °C at 10 °C/min.

Confirmation of the identity of TCDD was determined by responses from the sample in relation to responses from the standards and coincidence of retention time with that of the internal standard. The abundances of ions at m/z 320, 324, 257, and 259 relative to the base peak at m/z 322 usually should be ±10% of those abundances observed with standards for about the same amount of TCDD injected. The relative abundances of m/z 257 and 259 are ~50% of the base peak. The relative abundances of m/z 261 and 326 should be consistent with those of the standards but because of their low abundance are subject to greater variability. No interferences should be observed on ions at m/z 305, 307, and 321. The latter ion should show an approximately normal isotope abundance relative to m/z 320. The confirmation makes no distinction among the 22 possible isomers of TCDD.

Unlike the confirmation of identity which requires proper response of all ions, a quantitative measure can be made if an ion characteristic of native TCDD and an ion characteristic of the [^{13}C]TCDD are observed (e.g., m/z 322 and 334).

Table I. Resources Required To Extract and Clean Up Fish Samples

cleanup method	analysts set	no. of samples per set	extraction–cleanup time, h, per set[a]	extraction–cleanup time, h, per sample per analyst
FDA acid-base/HPLC	1	6	24	4
Dow dual-column/HPLC	2	4	16	8
EPA-A/B	2	4	8	4
EPA-Neut	1	4	8	2
FWS carbon/dual column	1	6	20	3.3
NYS multicolumn	1	2	16	8

[a] Time required for one or two analysts (see second column) to extract and clean up a set of samples.

Areas of peaks at m/z 320, 322, 332, and 334 were calculated by using the INCOS software. The amount of TCDD was quantitated as

$$\text{ppt of TCDD} = [(\text{area } m/z\ 332)/(\text{area } m/z\ 334)] \times [(\text{amount of } [^{13}\text{C}]\text{TCDD internal standard, pg})/(\text{amount of fish sample, g})]$$

The quantitation was checked for internal consistency by a similar calculation based on areas of m/z 320 and 332.

Full-Scan Gas Chromatography–Mass Spectrometry. The full-scan GC–MS analyses were carried out under the same chromatographic conditions as the MID GC–MS procedure. The MS conditions were altered to provide repetitive 2-s scans from mass 60 to 600 daltons at a sensitivity capable of detecting a response from the internal standard (m/z 332 and 334) (~200 pg) in order to determine the retention time of TCDD.

RESULTS AND DISCUSSION

The efficiency of the analytical cleanup procedures for TCDD in fish samples was determined by using the MID GC–MS procedure for TCDD. The results obtained from GC–EC and full-scan GC–MS are presented as additional measures of cleanup efficiency. The number of analysts per set, samples per set, and cleanup time per set reported by the participating laboratories are shown in Table I. The extraction–cleanup time, in hours per sample per analyst, ranged from 2 to 8; the EPA-Neut cleanup required the least time per sample (2 h), and the Dow and NYS cleanups required the most time per sample (8 h).

Electron Capture Gas Chromatographic Screening. Fish extracts from EPA-A/B, EPA-Neut, and NYS cleanup were not suitable for GC–EC screening because of the high levels of coeluting compounds (components with GC retention times at or near the retention time of TCDD) present in the extracts. Levels of coeluting components in extracts from the EPA-Neut cleanup were less than those encountered in extracts from the EPA-A/B cleanup, but the extracts were still unsatisfactory for GC–EC screening. FDA, Dow, and FWS cleanup procedures produced extracts acceptable for GC–EC screening to detect the apparent presence of at least 20–50 ppt of TCDD in the fish. Chromatograms from the FDA (both laboratories), Dow, and FWS reagent blanks (reagents taken through the procedure) were relatively free from coeluting components in the GC–EC. Reagent blanks from the BF/FDA, DET/FDA, and FWS cleanup gave GC–EC signals at the retention time of TCDD equivalent to 4–5 ppt of TCDD (20-g sample basis). The signal at the retention time of TCDD was not measured in the Dow

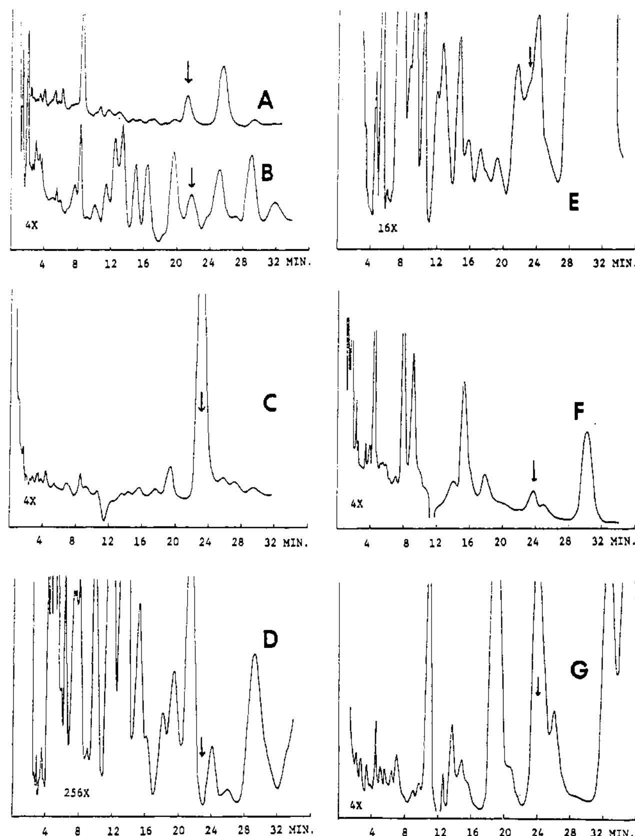

Figure 1. EC-GC chromatograms of extracts from unfortified catfish ($^{1}/_{20}$ of the sample extract). (A) BF/FDA; (B) DET/FDA; (C) Dow; (D) EPA-A/B; (E) EPA-Neut; (F) FWS; (G) NYS. The arrows indicate the retention time of TCDD, as determined by GC of a TCDD standard solution.

reagent blank extracts, since the [^{13}C]TCDD internal standard was added by Dow to the reagent blanks as well as to the samples.

Chromatograms of extracts from the unfortified catfish [sample 3 or sample 5 (BF/FDA)] are shown in Figure 1. GC-EC chromatograms from the various extracts give some indication of the relative efficiency of the individual cleanup procedures. The relatively high level of coextractives (undesired components present with TCDD in the final extract after extraction-cleanup) giving GC-EC responses in EPA (parts D and E of Figure 1) necessitated the use of 256× and 16× attenuation rather than the usual 4× attenuation in order to keep the chromatographic peaks on scale. The chromatogram of the extract from the Dow procedure is dominated by the response from the [^{13}C]TCDD internal standard but is otherwise relatively free from components giving GC-EC responses. The Dow cleanup appears by GC-EC to have the highest efficiency.

A comparison of TCDD recoveries from BF/FDA, DET/FDA, and FWS sample extracts is shown in Table II. Recoveries were calculated from the amount of TCDD determined in the fortified sample less the amount of TCDD determined in the unfortified sample. The unfortified sucker extracts gave GC-EC responses equivalent to 8-17 ppt of TCDD. The unfortified catfish extracts gave GC-EC responses equivalent to 26-45 ppt of TCDD. GC-EC responses detected in the reagent blanks, as mentioned above, were equivalent to 4-5 ppt of TCDD. Recoveries of TCDD from the fortified samples of sucker and catfish were in the range of 51-88% (average based on eight samples, 66%).

Multiple Ion Detection Gas Chromatography-Mass Spectrometry. The experimental data obtained with the GC-MS procedure using MID to analyze sample extracts consisted of 12 ion current chromatograms for each sample from each participant. Six ions were selected for illustration (m/z 257, 259, 320, and 322 of TCDD and m/z 332 and 334 of [^{13}C]TCDD) since they represented the principal ions of response for TCDD and [^{13}C]TCDD. These ion current chromatograms are shown in Figure 2 and represent responses observed for sample 4 (fortified catfish) obtained from the Dow cleanup procedure. This sample extract illustrates that TCDD elutes free of interferences (coextractives in the 10-15-s retention time window of TCDD) for all six ions illustrated (and for the other six monitored ions as well).

Table II. Added TCDD (ppt) Recovered through GC-EC Screening of Samples[a]

sample	BF/FDA	DET/FDA	FWS
(1) sucker	11	8	17
(2) sucker, fortified	73 (51)	66 (55)	*b*
(3) catfish	*b*	38	26
(4) catfish, fortified	131 (78)	104 (64)	90 (61)
(5) catfish	36	35	45
(6) catfish, fortified	143 (88)	117 (77)	83 (54)
(7) reagent blank	5	5	4

[a] Samples 2, 4, and 6 were fortified with 121 ppt of TCDD (BF/FDA samples) or 105 ppt of TCDD (DET/FDA and FWS samples). Percent recovery of added TCDD is in parentheses. [b] Some or all of the sample was lost.

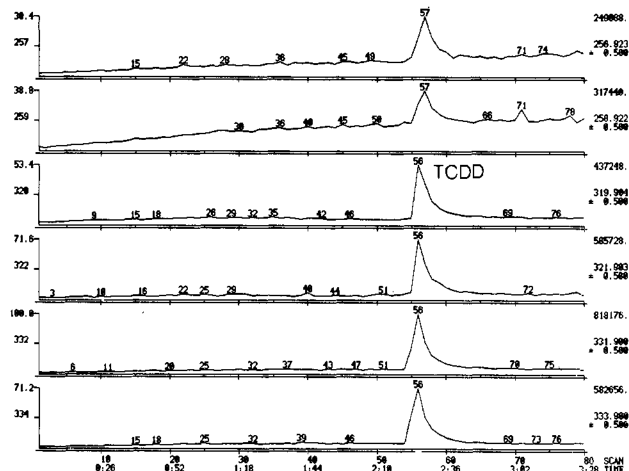

Figure 2. Six ion current chromatograms obtained by MID GC-MS (fortified catfish sample 4 from Dow cleanup procedure). Finnigan 3300F; electron impact, 24 eV; unheated source.

In the case of the BF/FDA cleanup procedure, samples were observed to have the envelope of response on ions m/z 259 and 261 which obscured the response from TCDD and therefore acted as an interference. The ion currents for the molecular ion cluster (m/z 320, 321, 322, 324, and 326) provided responses for TCDD which appeared to be free from interferences. These observations were generally applicable to the responses observed with samples prepared by the DET/FDA cleanup procedure.

In the case of samples from the EPA-A/B cleanup procedure, large background ion responses were observed during a significant part of the data acquisition for all 12 monitored ions. The background responses obscured the observation of a clearly defined TCDD response. Similarly, with the samples from the EPA-Neut cleanup, a large envelope of response on all 12 ions obscured the response from TCDD.

In the case of the Dow and FWS samples, the response from TCDD was the largest response observed on all six principal ions. This indicated an efficient cleanup by MID

Table III. Summary of MID GC-MS Results of Study of TCDD Extraction-Cleanup (Confirmation of Identity; Quantitation in Nanograms per Kilogram)[a]

sample no.[b]	BF/FDA		DET/FDA		NYS		EPA-A/B		EPA-Neut		Dow[c]		FWS	
	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.
1	no	5	no	6	no		no		no		no		no	9
2	no	67	no	89	yes	77	no		no		yes	67	yes	47
3	no	34	no	42	yes	57	no		no		yes	25	yes	22
4	no	188	no	99	yes	128	*d*	*d*	no		yes	113	yes	117
5	*e*	*e*	no	53	yes	38	*d*	*d*	*d*	*d*	yes	45	yes	56
6	no	178	no	199	yes	107	*d*	*d*	*d*	*d*	yes	100	yes	96

[a] Confirmation of the identity of TCDD is obtained if the responses of the 12 monitored ions for the sample extract are consistent with the responses of the 12 monitored ions of the TCDD standard. Quantitation is based on the observed responses at m/z 322 and 334. [b] See Table II for sample identity. [c] Quantitation by the external standard because of the [^{13}C]TCDD carrier. [d] Samples were not analyzed due to large amounts of coextractives. [e] Some or all of the sample was lost.

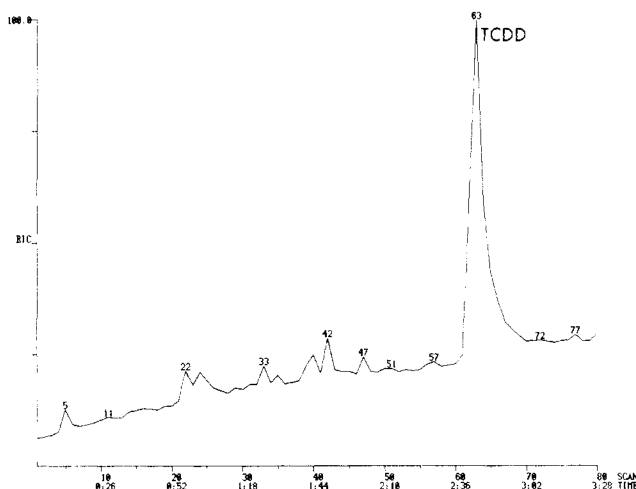

Figure 3. Reconstructed ion chromatogram of 12 ions obtained by MID GC-MS (fortified catfish sample 4 from FWS cleanup procedure).

GC-MS. The NYS samples provided a clear indication of the TCDD response as well, but significant responses were also observed from coextractives during the data acquisition. Analysis of all 12 ion chromatograms for all participants indicated that only the NYS, Dow, and FWS cleanup procedures provided sample extracts with no interferences at the retention time of TCDD.

As an additional illustration of the data, a reconstructed ion chromatogram that represents the sum of all 12 ion currents is illustrated in Figure 3. The reconstructed ion chromatogram is shown for sample 4 (fortified catfish, FWS cleanup) and emphasizes the high cleanup efficiency observed with the FWS procedure. In general, all cleanup procedures yielded higher levels of coextractives for catfish samples than for sucker samples.

A summary of the results obtained by MID GC-MS analysis of all sample extracts is given in Table III, which shows whether the identity of TCDD was confirmed in the sample and the quantitation of observed responses for ions at m/z 322 and 334 as described earlier. In some instances, quantitation based on m/e 322 was possible even though a confirmation of identity was not achieved because of interferences on other ions.

On the basis of the MID GC-MS data, extracts obtained by using the six extraction-cleanup procedures may be placed into four categories. In the first category are the Dow and FWS procedures. TCDD identity was confirmed and its level quantitated in all fortified sucker and catfish samples and both unfortified catfish samples (no. 2-6). TCDD identity was not confirmed in the unfortified sucker (no. 1), but a quantitative value was based on the observed response at m/z 322 as provided in the procedure. Ion current chromatograms for the 12 monitored ions indicated that these extracts were free of interferences and were free of coextractives throughout the acquisition time interval (2-3 min). In the second category is the NYS procedure. As was the case in the first category, TCDD identity was confirmed and the level quantitated in samples 2-6. However, ion chromatograms indicated the presence of significant amounts of coextractives in the acquisition time interval.

The FDA procedure (used by BF/FDA and DET/FDA) was in the third category. TCDD identity was not confirmed in the six samples due principally to interferences in the form of envelopes on m/z 259 and 261 (the $M^{+\cdot} - COCl\cdot$ ions). The overall levels of coextractives appeared significant in the acquisition time interval based on the 12 monitored ions. The molecular ion cluster generally appeared free from interference although some quantitative results appear too large (greater than 100% recovery of added TCDD).

The fourth category includes the EPA-A/B and EPA-Neut procedures. Sample extracts from both of these procedures contained large amounts of coextractives in the acquisition time interval. Also, interferences were observed on all 12 ions in the retention time window of TCDD. Consequently, these samples could not be confirmed as containing TCDD nor could quantitation be based on m/z 322 and 334.

As an example of the mass spectrum obtained from a fortified sample, Figure 4A illustrates the MID GC-MS spectrum for sample 4 (fortified catfish) obtained from the Dow cleanup procedure. The spectrum for TCDD in the sample can be compared to the spectrum of a standard shown in Figure 4B. The relative abundance of m/z 332 is greater than that of m/z 334 for the sample (Figure 4A), indicating that the Dow [^{13}C]TCDD is not fully labeled. This conclusion is supported by full-scan GC-MS data. In fully labeled [^{13}C]TCDD, m/z 334 is the base peak. Figure 4C illustrates the spectrum of TCDD in the unfortified catfish (sample 3) obtained from the FWS cleanup procedure; in this spectrum the relative abundances of m/z 332 and 334 of the internal standard are appropriate for a tetrachlorinated compound. As indicated in Table III, the presence of TCDD was confirmed in extracts from both unfortified catfish samples (no. 3 and 5) obtained from the NYS, Dow, and FWS cleanup procedures.

Full-Scan Gas Chromatography-Mass Spectrometry. Sample extracts were also examined by full-scan GC-MS from m/z 60 to m/z 660. Under these conditions, ions at m/z 129 and 149, due to coextractives, were frequently observed. A relatively efficient cleanup procedure such as the Dow or FWS procedure provided relatively weak responses for these ions and others throughout the mass range scanned but a clearly defined response for

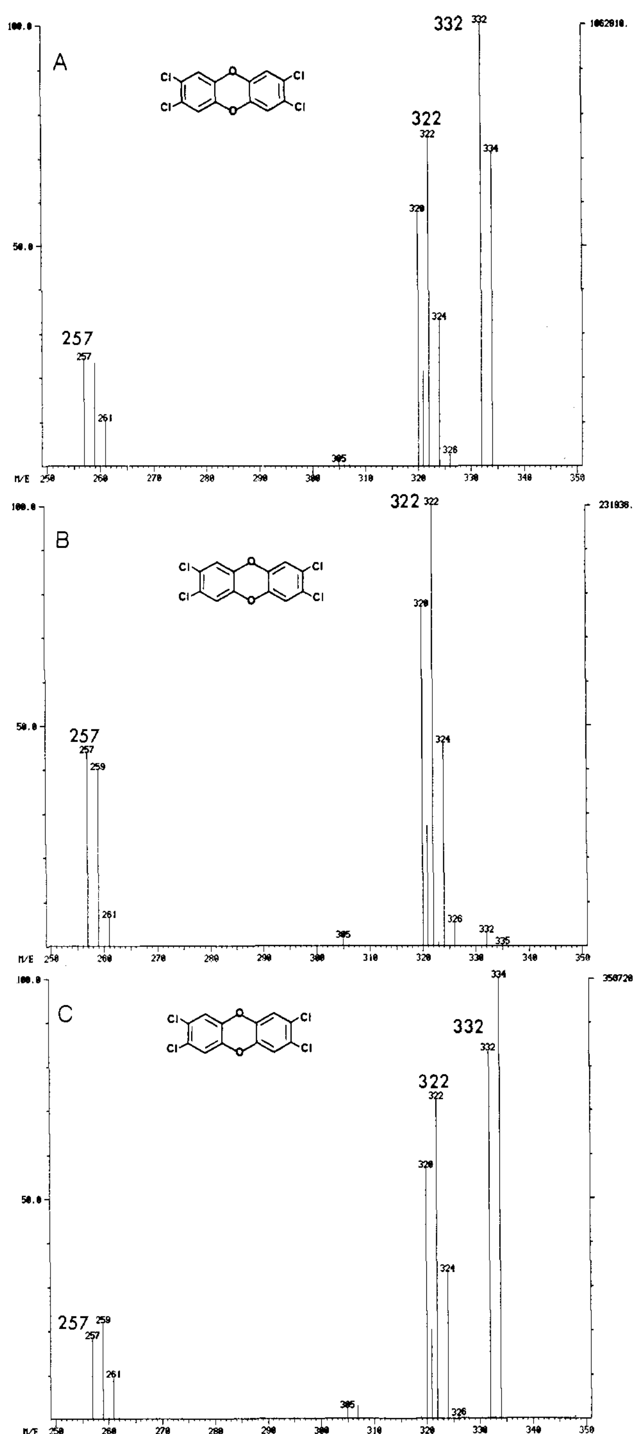

Figure 4. Background-subtracted spectra obtained by MID GC–MS. Finnigan 3300F; 24 eV; unheated source. (A) Sample 4 (fortified catfish) obtained from Dow cleanup procedure; (B) 58 pg/0.5 μL of the TCDD standard; (C) sample 5 (unfortified catfish) obtained from the FWS cleanup procedure.

[^{13}C]TCDD (m/z 334). In the case of the extracts from EPA-A/B and EPA-Neut cleanup, the [^{13}C]TCDD response was obscured by interferences. The TCDD responses were small in the full-scan mode since the amount of TCDD present was of the order of 100 pg. In general, the full-scan GC–MS data reinforced the conclusions reached from the data obtained by the MID GC–MS procedure. The Dow and FWS procedures provided the least amount of response for coextractives in the full-scan mode of operation. The EPA cleanup procedures provided extracts exhibiting large responses due to interfering compounds.

In conclusion, the efficiency of six extraction–cleanup procedures was determined by an MID GC–MS procedure for confirming the identity of and quantitating TCDD in fish. The cleanup procedures were characterized in terms of interferences to TCDD and coextractives observed within the acquisition time interval. The cleanup procedures were placed into four categories, with the Dow and FWS procedures representing the most efficient procedures since no interferences and low levels of coextractives were observed. In the second category was the NYS procedure, which gave extracts exhibiting no interferences but significant levels of coextractives. The third category included the FDA procedure, which exhibited some interferences but significant levels of coextractives. The EPA-A/B and EPA-Neut procedures, which afforded extracts which contained large amounts of interferences and coextractives, were in the fourth category. These conclusions were substantially supported by two additional measures of the relative cleanup efficiency. Screening by GC–EC indicated that the Dow cleanup was the most efficient. Full-scan GC–MS data fully supported the findings obtained by MID GC–MS.

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Crystal Structure of (±)-Methyl 2-[4-(2,4-Dichlorophenoxy)phenoxy]propionate, a New Selective Grass Herbicide of the Phenoxy–Phenoxy Series

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The crystal structure of the selective phenoxy–phenoxy grass herbicide (±)-methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propionate (Diclofop-methyl) has been determined by direct methods and refined by least squares to a final residual of 0.039 for 1297 observed reflections. The conformational features of the 2-phenoxypropionate moiety in the molecule closely resemble those of the substituted 2-phenoxypropionic acid herbicides, where structural aspects are well systematized. There appears to be little conformational change associated with the presence of the substituent methyl ester group on the acid.

The title compound, (±)-methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propionate (compound HOE 23 408; proposed common name Diclofop-methyl) is the first commercial member of a new series of phenoxy–phenoxy herbicides developed from 1971 onward by Hoechst AG, Frankfurt-Main, Germany) (Nestler et al., 1979). It has also been shown that the (+) enantiomer is the herbicidally active species (Nestler and Bieringer, 1980). Now marketed under the trade names Illoxan, Hoelon, and Hoe-Grass, this compound (along with other members of the series) has specific herbicidal properties, quite unlike those of the synthetic auxin compounds of the 2,4-D type, in particular, the 2-propionic acids such as Dichloroprop, Silvex, and Mecoprop. Optimum activity in the series is attained in Diclofop-methyl, being remarkably effective against grassy-type weeds such as wild oats and millet, which constitute a serious pest problem in commercial crops, e.g., wheat, barley, sugar beet, and soybeans. It is tolerant toward many monocotyledons and dicotyledons. Just as with the phenoxyalkanoic acid series, activity is found in both the free acid salts and esters.

Development of the series involved a comparison of likely compounds variously substituted in the second aromatic ring with the inactive nonsubstituted parent compound. This was done by using the Topliss operational scheme for aromatic substitution (Topliss, 1972). In this circumstance it was of interest to compare the solid-state structure of Diclofop-methyl with those of the phenoxy acid analogues. Altogether structural and conformational aspects of 20 phenoxyalkanoic acids have been determined by using X-ray diffraction techniques (Table I). Two reviews of the structural trends of these acids (Smith and Kennard, 1979; Kennard et al., 1981a) indicate that the preferred conformation of the acetic acid analogues is one with the gross molecule planar, whereas for 2-propionic acids, the α-substituted methyl group tends to lie in the appropriate molecular plane with the oxo acid side chain in a perpendicular orientation. This is found in two commercial propionic acid herbicides whose structures have been determined [Silvex (Smith et al., 1977); Mecoprop (Smith et al., 1980)]. Furthermore, these acids are chemical analogues of compounds having hypolipidaemic properties, e.g., 2-[4-(4-chlorophenoxy)phenoxy]propionic acid (Schacht, 1977). An investigation of the structural systematics of this series has now been initiated (Kennard et al., 1981a).

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EXPERIMENTAL SECTION

Crystal Data. $C_{16}H_{14}Cl_2O_4$; M_r = 341.2; monoclinic; space group $P2_1/c$; a = 13.968 (5) Å, b = 12.561 (6) Å, c = 9.247 (5) Å; β = 98.74 (4)° (cell parameters and their standard deviations were obtained from 12 high-angle reflections by using the Syntex system); V = 1603 Å^3; Z = 4; D_{calcd} = 1.41 g cm^{-3}; $F(000)$ = 704; μ(Mo Kα) = 4.04 cm^{-1}.

Data Collection, Structure Solution, and Refinement. Crystals suitable for X-ray analysis were obtained by recrystallization from hexane of the sample of HOE 23 408 provided by Hoechst Aktiengesellschaft, Frankfurt. A total of 1297 reflections with $I > 2.5\sigma(I)$ were considered observed out of 2025 collected in a unique set from a crystal (0.22 × 0.13 × 0.35 mm) mounted about the c axis on a Syntex $P2_1$ four-circle diffractometer ($2\theta_{max}$ = 45°) by using graphite crystal monochromated Mo Kα radiation (λ = 0.7107 Å). No corrections were made for absorption [μ(Mo Kα) = 4.04 cm^{-1}].

The structure was solved by using MULTAN (Germain et al., 1971). Full-matrix least-squares refinement with anisotropic thermal parameters for all nonhydrogen atoms reduced R [$=\sum||F_o - F_c||/\sum|F_o|$] to 0.039 and Rw

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