Journal of Chromatography, 478 (1989) 349–367
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 644

MULTIRESIDUE PROCEDURES FOR THE DETERMINATION OF CHLORINATED DIBENZODIOXINS AND DIBENZOFURANS IN A VARIETY OF FOODS USING CAPILLARY GAS CHROMATOGRAPHY–ELECTRON-CAPTURE DETECTION

JAMES S. JASINSKI

U.S. Department of Health and Human Services, Food and Drug Administration (Detroit District), 1560 E. Jefferson Avenue, Detroit, MI 48207 (U.S.A.)

(First received February 10th, 1989; revised manuscript received May 25th, 1989)

SUMMARY

Multiresidue digestion–extraction procedures for the determination of chlorinated dioxins and furans in a wide variety of products are presented. Procedure selection is dependent upon the residue(s) of interest, and on the fat content of the product. Additional cleanup is accomplished using column chromatography and a Florisil trap. The separation of residues is achieved by fraction collection off of two high-performance liquid chromatographic systems. Capillary gas chromatography employing electron-capture detecton is used for quantitation. The extracts are suitable for gas chromatography–mass spectrometry or gas chromatography with Hall electrolytic conductivity detection. Results of analysis, recovery data, and interlaboratory comparisons are presented. Spike recoveries will typically average 90% ± 10%.

INTRODUCTION

The occurrence of polychlorinated dibenzodioxins (PCDDs) in the environment has presented a formidable challenge to the residue chemists who have had to devise procedures for their analyses at part per trillion[a] (ppt) levels. Most of the procedures have been developed for the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD)[1–4]. Other procedures have been developed for the higher chlorinated dioxins, and for the polychlorinated dibenzofurans (PCDFs)[5–8]. In general the method of choice for these procedures involve some sample pretreatment followed by determination with gas chromatography–mass spectrometry (GC–MS). Albro *et al.*[9] reported the results of an interlaboratory study involving the determination of PCDDs and PCDFs in human adipose tissue. Eight different laboratories participated in the study, each using their own procedure, and all of which used GC–MS for quantitation of unspiked and spiked sample portions. The results indicated that the procedures were qualitatively reliable. The quantitative correlation of sample and recovery data was

[a] Throughout this article, the American billion (10^9) and trillion (10^{12}) are meant.

0021-9673/89/$03.50

not as obvious. Through an evaluation of the data the authors identified significant problem areas which resulted in the scatter of the data. These included: (1) the application of procedures developed for 2378-TCDD which were inappropriate for the higher PCDDs and PCDFs, (2) the use of procedures which had not been tested on a wide variety of sample matrices such as adipose tissue, and (3) the differences in capabilities of the GC–MS systems used in the determinative step.

Our laboratory does not have a GC–MS unit which can be dedicated to dioxin analysis since it must service a variety of analytical disciplines. As a matter of practice, we prefer to reserve the GC–MS technique for confirmation only. Consequently, we require a completely different technique for primary quantitation, namely GC–electron-capture detection (ECD). In addition, we are involved with a wide variety of sample types, the analysis of which includes both the PCDDs and PCDFs. Obviously, we needed a comprehensive procedure applicable to a wide variety of products which is sensitive, quantitative and isomer specific.

The procedure of Niemann *et al.*[10], developed for 2378-TCDD in fish, has been used extensively in our laboratory along with the use of 1378-TCDD as an internal standard[11]. Several additional steps were developed by this laboratory in order to improve spike recoveries through the procedure, to get cleaner sample extracts prior to injection on the high-performance liquid chromatographic (HPLC) systems, and to include a variety of different sample types. The procedure was expanded to include the higher PCDDs and the PCDFs. Fractionation of sample extracts utilizing the C_8/C_{18} HPLC systems allowed for the isomer specificity needed for all of the PCDDs studied. Additional specificity was supplied by the capillary GC–ECD determinative step.

The inclusion of a Florisil trap resulted in extracts which were two orders of magnitude cleaner, upon direct injection into the C_8 HPLC system, than the fraction obtained from the size-exclusion HPLC (HPSEC) system originally used. This allowed the elimination of the HPSEC step and resulted in considerable savings in analytical time and equipment. Florisil had previously been used for sample cleanup[12], but not spcifically as a trap. The Florisil allows for unlimited washing of the extract with hexane while completely retaining all of the PCDDs and PCDFs. The PCDDs and PCDFs are then eluted off the Florisil with methylene chloride.

A methylene chloride extraction was developed for the transfer of residues between the two HPLC systems to replace the original benzene extraction. The methylene chloride extraction has proven to be more reproducible and complete.

With the addition of specific digestion and extraction procedures based on the desired residues, and on the fat content of the product, a comprehensive procedure is presented for the analysis of PCDDs and PCDFs in a wide variety of products.

EXPERIMENTAL

Safety

All laboratory personnel should be aware of the extreme toxicity of these compounds and take every precaution to prevent exposure of these residues to themselves and to others. Training should be provided in the safe handling of these materials, and in the safe disposal of their wastes. The acid-coated silica used in this procedure should be treated as a concentrated acid and also as a potental inhalation hazard.

Reagents and solvents

Water is prepared by passing previously deionized water through a Millipore or similar water purification system. All solvents used are of UV- or HPLC grade obtained from Burdick and Jackson, or EM Science. Florisil (Fisher F-101 or equivalent) is prepared by washing with twice its volume of hexane and methylene chloride. The Florisil is activated and stored in a 125°C oven. All other chemicals are of ACS reagent grade and used without further purification. All glassware is scrubbed with hot detergent, followed by rinsing sequentially with tap water, deionized water, and acetone.

Digestion solution and chromatographic supports

The digestion solution is a 40% (w/v) KOH in water. The digestions are performed in a 120-ml, glass bottles equipped with PTFE-lined screw-caps. The preparation of 44% sulfuric acid on silica, and 33% 1 *M* KOH on silica has been previously described[13,14].

Standards

2378-TCDD at 67.8 ng/ml in isooctane was obtained from the National Bureau of Standards as a Standard Reference Material. Other dioxin congeners were obtained from Niemann *et al.*[10], who performed the purity testing of these compounds. The remaining dioxins and furans were obtained commercially through Cambridge Isotopes.

For GC–ECD, a 200-pg/μl 1378-TCDD solution is prepared by serial dilution of a 1-ng/μl stock solution in isooctane. The 2378-TCDD solution is prepared at a nominal 10 pg/μl in isooctane. Mixed standards of the higher chlorinated dioxins are also prepared in isooctane ranging from 2 pg/μl for the penta-, to 5 pg/μl for the octaisomers. The PCDFs are also prepared in isooctane at similar concentrations. Mutual overlap of retention times for some of the higher congeners of the dioxins and furans precludes the preparation of mixed dioxin–furan standards for the hepta- and octa-congeners.

For HPLC the standards used to set the collection windows are made to contain 1 to 1.5 ng/μl of the individual component in the same solvent used to dissolve the sample. Detector sensitivities of 0.01 to 0.02 AUFS are normally used to set the collection windows.

The acid–silica column is prepared by packing a 30 × 2 cm glass column sequentially with layers of 1 g silica, 2 g 33% 1 *M* KOH on silica, 1 g silica, and finally 10 g 44% sulfuric acid on silica. The column is packed by gentle tapping after each addition. The Florisil column is prepared by adding 2.0 g of hot Florisil to a 25 × 1 cm glass column, gently tapping the column, and quickly covering the Florisil with hexane.

The prepurified nitrogen used for evaporations must be further purified. The assembly of this apparatus has been previously described[13].

Separate C_8 and C_{18} HPLC systems were set up as previously described[10]. The C_8 system was eluted at 45°C with acetonitrile–water (75:25, v/v) at 2.4 ml/min. A reservoir containing 100% acetonitrile was connected to another port of the solvent selection switch on the pump to allow for washing of the column after window collection. The C_{18} system was eluted at 45°C using acetonitrile at 1.2 ml/min. Solvent

reservoirs were continuously sparged with helium during use. UV detection at 235 nm is recommended for both systems. All injections were made with 100-μl syringes equipped with PTFE-tipped plungers.

All GC–ECD separations were performed on the J & W DB-1, fused-silica columns, 60 m × 0.25 mm, 0.25-μm film. A Varian 6000 GC–ECD system equipped with an on-column injector, and a Varian 3700 GC–ECD system equipped with a splitless injector were used in this study. Both instruments were equipped with constant-current, pulse-modulated, ^{63}Ni electron-capture detectors operated at 350°C. On-column injections were made at 170°C and then ramped at 140°C/min to 310°C and held for 15 min. Splitless injections were made at 310°C using the timed sequence described earlier[10]. Similar column conditions are used in both instruments. Nitrogen is used as the makeup and/or purge gas, and flows are adjusted for optimum response. After injection the column oven temperature is held at 75°C for 2 min, then programmed to 195°C at 25°C/min, then to 310°C at 5°C/min and held for 2 min. This program adequately separates all of the residues studied, and can be modified for other desired residues. The hydrogen carrier gas supplied to the instruments is passed through a series of traps containing activated charcoal, molecular sieve, and an oxygen scrubber in that order. The nitrogen is also passed through a similar trap system except that a furnace type gas purifier is installed in the line between two molecular sieve traps.

Sample preparation

(a) Fish samples are filleted and skinned. The fillets are passed through a meat grinder three times, mixing between each pass through the grinder.

(b) Meat and fatty tissue are deboned, and treated the same as the fish above. Samples of fat are heated on a steam bath until clarified and mixed.

(c) Egg samples are shelled and blended at moderate speed. Milk samples are blended similarly.

(d) Viscous samples are manually mixed.

(e) Dry products are ground in a suitable mill to pass a 1.0-mm screen.

(f) Sediments are dried under moderate heat (60°C) and ground in a motorized mortar and pestle to a powder. The moisture loss is determined for each sample so that results can be calculated back to the wet basis.

All analytical sample portions are weighed immediately after compositing, especially for samples containing both oil and water. Reserve portions are frozen for storage. If additional analysis is required, the frozen samples are thawed and reblended to ensure homogeneity in the analytical portion taken.

Digestion and extraction procedures

According to the procedure of Niemann[11], 20 ng of 1378-TCDD is added to each sample as an internal standard (I.S.). All 2378-TCDD results are corrected for the I.S. recovery. A reagent blank, which also contains the I.S., accompanies each set of samples.

Dioxins in high- and low-fat samples. (a) Weigh a 20.0-g sample into a digestion bottle and add 100 μl 1378-TCDD standard. Add 40 ml of KOH solution and 20 ml ethanol, stopper and mix. Place bottle on a mechanical shaker and shake for 3 h at room temperature. Transfer the digestate to a suitable separator using a 20-ml hexane rinse. Shake vigorously for 1 min and allow the layers to separate.

(b) If only two layers are visible at this point, drain the lower aqueous layer into a second separator, and transfer the hexane layer into the original bottle with the aid of a 2 ml hexane rinse. This procedure is normally applicable to products of intermediate fat content such as liver, eggs, milk and fish. A 125-ml separator is adequate for the extraction. Repeat the extraction with three 20-ml portion of hexane. After the last extraction, discard the aqueous layer and combine all the hexane extracts in the last separator with several 3-ml hexane rinses. Gently rinse the combined extracts with two 25-ml portions of water and discard the water. Add 25 ml concentrated sulfuric acid slowly and carefully to start, allowing time for the reaction to subside and cool. Shake well and allow to set for at least 1 h. Break any emulsions by carefully adding small increments of water. Drain and discard the acid layer. Repeat the acid treatment until the upper hexane layer is clear and colorless, and the lower acid layer is moderately brown. Reserve the hexane extract for the acid–silica column.

(c) If more than two layers are visible after the first hexane extraction, allow the separator to set for several hours to allow the lower aqueous layer to separate as much as possible. This procedure is usually required for high-fat products. A 250-ml separator is required for this purpose. Drain the aqueous layer into a second separator, and transfer the organic layers into the original digestion bottle. Extract the aqueous layer with an additional $3 \times$ 20 ml hexane, combining the hexane extracts with the organic layers. Discard the aqueous layer and combine all the hexane extracts in the last separator. Wash the combined layers with an additional 100 ml of KOH solution and allow to separate several hours. Drain and discard only the clear aqueous layer. Carefully add 25 ml of concentrated sulfuric acid, shake frequently for 1 h, and treat as in (b) above.

Dioxins in non-fatty products. (a) Honey and skimmings: treat 20 g as in (a) and (b) of the section *Dioxin in high- and low-fat samples* above.

(b) Treated wood: extract 5.0 g of shavings with 20 ml ethanol overnight and treat as in (a) of the section *Dioxin in high- and low-fat samples* above. Filter through glass wool and wash filter with 20 ml hexane when transferring to separator. Treat as in (b) of the section *Dioxin in high- and low-fat samples* above.

(c) Rice: extract 20 g ground rice with 50 ml methylene chloride for 3 h and filter through glass wool with rinsing. Evaporate the solvent and dissolve residue in 100 ml hexane.

(d) Gelatin: treat 20.0 g as in (a) and (b) of the section *Dioxin in high- and low-fat samples* above. Warm the digestion bottle slightly if solidification occurs.

(e) Sediments and soils: use the procedure of Albro *et al.*[6]. Sandwich 2–20-g portions of dried and ground sample between 15-g layers of sodium sulfate in a suitable column. Moisten with acetone, and elute with 50 ml ethyl acetate, and then 100 ml methylene chloride. Evaporate the solvent to dryness and dissolve the residue in 10 ml hexane.

(f) Chemical wastes: a suitable quantity of waste is mixed with 50 ml water in a 125-ml separator and extracted with three 25-ml portions of methylene chloride. The solvent is evaporated and the residue dissolved in 100 ml of hexane.

Furans and dioxins in high- and low-fat samples. As will be discussed later, the furans are not stable in the presence of base. This precludes the use of an alkaline digestion. Acid digestions have been used for their determination[15,16]. The following procedure using 80% sulfuric acid, although somewhat tedious, has been used successfully in our laboratory for both furans and dioxins.

The recommended amount of sample to be analyzed depends on the amount of fat present in the sample. For samples containing less than 20% fat, 20 g can normally be analyzed. For samples with 25–70% fat, only 10 g is recommended. For higher fat levels, 5 g is recommended.

Procedure: mix the sample portions with 100 ml hexane in a 250-ml separator. Cautiously add 25 ml 80% sulfuric acid and shake frequently for 1–2 h. Break any emulsions with small amounts of water and drain the acid layer. Repeat the procedure with additional portions of acid until the hexane layer is clear and the acid layer is not appreciably darkened after 1 h. After the bulk of the sample has been reacted, concentrated acid can be used to speed up the digestion. The initial use of concentrated acid leads to excessive charring of the sample. Emulsions are broken with small amounts of water. The hexane extract is then saved for the acid–silica treatment.

Furans and dioxins in non-fat samples. (a) The furans, as well as the dioxins, can be determined in all of the hexane extracts obtained under the section *Dioxins in non-fatty products* that did not involve alkaline digestion.

(b) Honey and other carbohydrates: dissolve 20.0 g sample in 60 ml water in a 125-ml separator and extract with three 25-ml portions of methylene chloride. Evaporate the combined extracts to dryness and dissolve the residue in 100 ml hexane.

(c) Treated wood: extract 5.0 g of shavings with 50 ml methylene chloride for 3 h on a mechanical shaker. Quantitatively filter the extract through glass wool. Evaporate the solvent to dryness, and dissolve the residue in 100 ml hexane.

Column chromatography

An acid–silica column is prepared and prewashed with 40 ml of hexane. After washing, the acid–silica column is mounted directly over a Florisil column so that the effluent from the acid column passes through the Florisil. Pass the sample extracts in hexane completely through both columns, followed by two 20-ml hexane rinses. Remove and discard the acid column, and rinse the Florisil column with an additional four 20-ml portions of hexane. Discard all of the hexane rinses. Elute the dioxins and furans off of the Florisil with 20, 20 and 10-ml portions of methylene chloride, collecting the eluates in a 250-ml Florence flask. The methylene chloride is conveniently evaporated by adding a few carborundum boiling chips and placing the flask on the metal edge of a covered steam bath, and brought carefully to a low boil. Care must be taken not to let the flask get too hot or loss of sample may occur. Alternatively, Kuderna-Danish equipment may be used for this concentration step using a 60°C water bath. When the volume of solvent approaches 1 or 2 ml, remove the flask, allow to cool, and transfer the extract to a 10-ml Mills tube with the aid of three 2-ml methylene chloride rinses. Evaporate the extract to dryness under nitrogen. Then rinse the walls of the tube sequentially with 250, 150, 100 and 50-μl portions of methylene chloride, evaporating each rinse to dryness separately. The purpose of the rinsing is to concentrate the residue in the bottom 100 μl of the tube. Dissolve the residue in 40 μl of acetonitrile–methylene chloride (75:25, v/v). Fill a 100-μl syringe with the extract along with three 20-μl rinses, and inject the sample extract into the C_8 HPLC system.

High-performance liquid chromatography

The C_8 and C_{18} HPLC cleanup systems used have been reported by other investigators[10], and have been expanded to include the other PCDDs and the PCDFs.

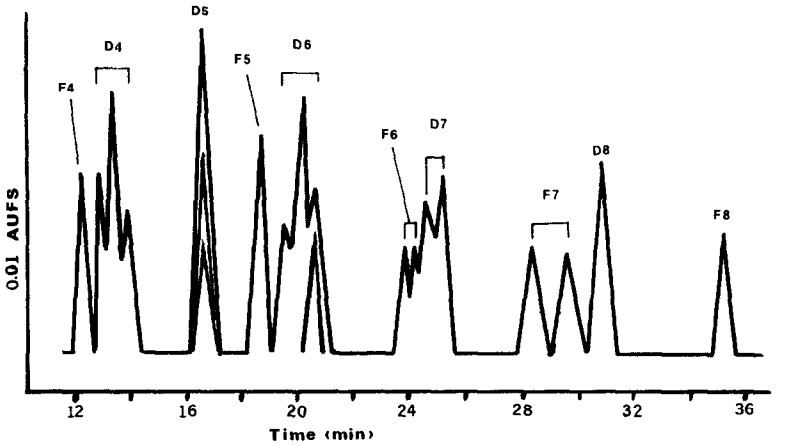

Fig. 1. The HPLC separation of a mixture of dioxin and furan standards on the C_8 system. See text for experimental conditions. The dioxin and furan peaks are identified by D or F respectively, followed by a number indicating the amount of chlorine substitution. The lower, single peaks represent the response of isomers injected individually.

Of the two, the C_8 system offers the greater resolution and variability of window selection as seen in Fig. 1, 2 and 3, which are partially redrawn. Fig. 4 represents actual chromatograms obtained from the injection of a mixed dioxin standard on both HPLC systems. Windows are set by the injection of appropriate, concentrated standards. The time of peak onset and return are measured with a stopwatch and normally 15 s are added to either side of the selected window. The standard solvent should be the same as that used for the samples, and the standard aliquot taken should be diluted to the same injection volume as that for the sample. After the injection of concentrated standards, the injectors should be backflushed. At low screening levels no dioxin peaks are

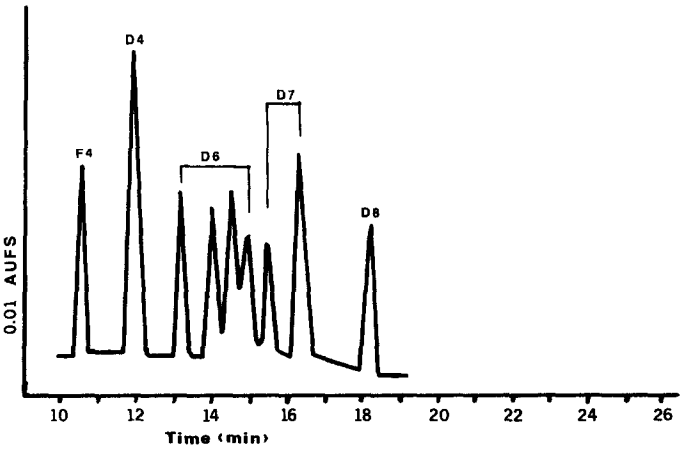

Fig. 2. The retention of dioxin standards on the C_{18} HPLC system. See text for experimental conditions. The standard peaks and amount of chlorine substitution are identified as in Fig. 1.

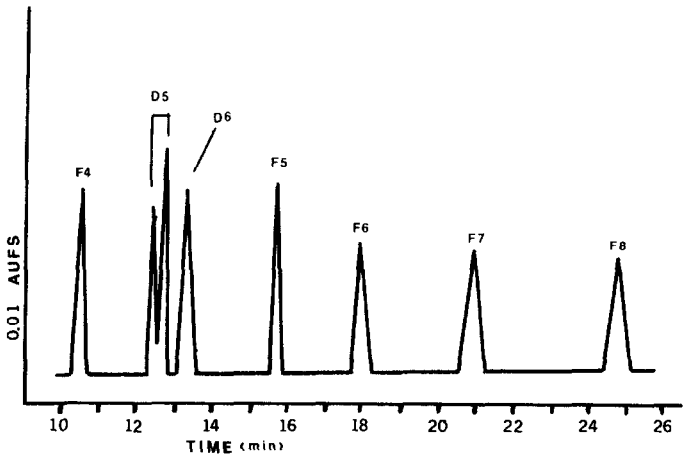

Fig. 3. The retention of a standard mixture containing mostly furan congeners on the C_{18} system. All of the congeners contain 2,3,7,8-chlorine substitution, and are identified as in Fig. 1.

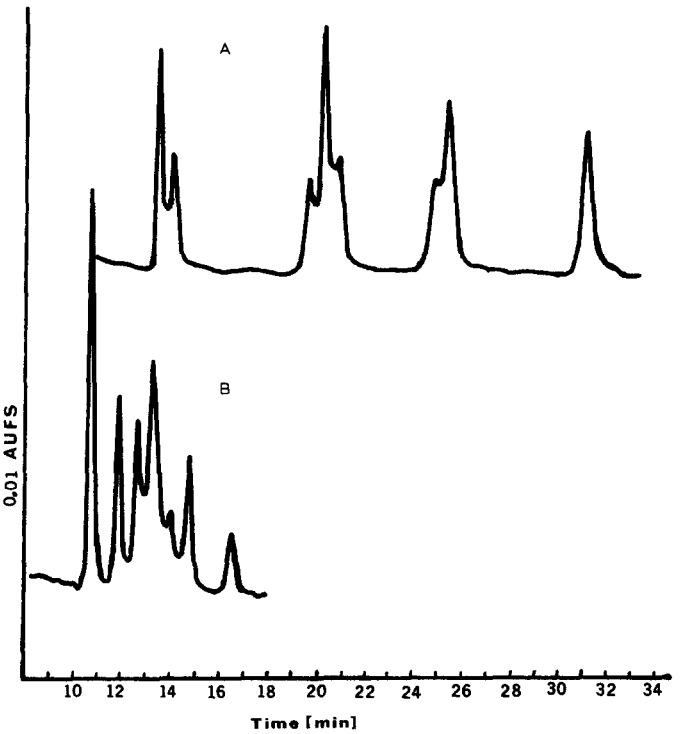

Fig. 4. Actual HPLC chromatograms for a standard mixture containing nine dioxins on (A) the C_8 system and (B) the C_{18} system.

normally observed in the sample chromatogram. Only the internal standard peak can be seen if not obscured by sample background.

Inject the sample extract into the C_8 system and at the proper time, manually switch the column effluent to a separator and collect the window, switching the effluent back to waste at the end of the window. The separator must be large enough to allow for a minimum 1:4 dilution of the collected volume. Some samples will have late eluting peaks which can be washed off of the column by switching the mobile solvent to 100% acetonitrile for about 20 min, and then switching back.

Dilute the collected window at least 1:4 by volume with an aqueous 2% sodium bicarbonate solution saturated with methylene chloride, and extract with three 2-ml portions of methylene chloride saturated with bicarbonate solution. Collect the extracts in a clean Mills tube, and evaporate to dryness under nitrogen. Wash down the sides of the tube with methylene chloride as before, concentrating the residue in the lower portion of the tube.

The sample is ready for injection into the C_{18} system. The procedures for window selection mentioned for the C_8 system are applicable here. Dissolve the residue in 40 μl of acetonitrile and draw into a 100-μl syringe followed by three 20-μl rinses. Inject the sample and collect the desired windows in a suitable Mills tube, the size of which depends on the volume of the window collected. The hepta- and octa- furans must be collected in a separate window since they interfere with the dioxins in the GC–ECD determination. Evaporate the solvent and concentrate the residues as before.

Capillary gas chromatography

Initially dissolve the residue in 15 μl of toluene added from a 100-μl syringe. Add 85 μl of isooctane to give a total volume of 100 μl. Based on a 20-g sample size, a 1-μl injection of this solution represents 0.2 g of sample. Quantitation is accomplished by comparison with a chromatogram of a similarly injected standard mixture containing the appropriate residue(s). In order to minimize any error due to detector non-linearity, all significant residues are quantitated by matching the response of the sample and standard chromatograms as closely as possible. Recoveries and control samples are calculated the same way. Most of the presented data are based on peak height measurements.

RESULTS AND DISCUSSION

KOH digestion

The KOH digestion originally used by Baughman and Meselson[17] generally does an excellent job of saponification. It has been reported that PCDDs are degraded in the presence of hot alkali, however, based on the recoveries obtained in this study, no significant degradation appears to occur with a 3-h room temperature digestion. PCDDs have also been reported to be light sensitive[12]. Therefore all dioxin and furan solutions are protected from light as much as possible, especially in aqueous solution.

Conversely, the PCDFs are degraded by alkali with the amount of degradation increasing with the increasing molecular weight of the congeners. In a fish matrix, the amount of degradation varies from 12% for 2378-TCDF, to 89% for octachloro-dibenzofuran. In the absence of a sample matrix, the degradation is even more

pronounced. As a result, the PCDFs can be determined by using only neutral or acid pretreatments.

For most samples, the KOH digestion is adequate for a 20-g sample size, and after elution from Florisil, there is usually no visible fat residue in the Mills tube. On rare occasions, certain high-fat samples, such as hamburger, can present problems due to the quantity and type of fat present. Using a 20-g sample size, some fat occasionally passes through the Florisil column. Even though the quantity is small (approximately 200-μl), this is too much to inject into the C_8 HPLC system. If this occurs the analysis must be repeated using a smaller portion of sample.

Trap evaluations

Activated alumina has been used to clean samples for 2378-TCDD[17]. Therefore an attempt was made to determine its suitability as a trap for all the dioxins. A mixture of eight dioxin standards in 60 ml of hexane were put on a 1.0-g alumina column and allowed to drain. The column was then eluted with separate 20-ml portions of methylene chloride and analyzed separately. Only 2378-TCDD and octachlorodibenzodioxin (OCDD) were quantitatively retained. Varying amounts of the hexa- and hepta-congeners washed through the column with the hexane with losses ranging from 8 to 69%. Alumina was deemed unsuitable for use as a trap.

Florisil had also been used[12] for the cleaning of samples using mixtures of hexane and methylene chloride or chloroform. The procedures worked for 2378-TCDD, however the break-through volume of the dioxin had to be determined beforehand. This was time-consuming and was based on the assumption that the sample matrix had little effect on the break-through volume. A study was therefore initiated to investigate the use of Florisil strictly as a trap for dioxins using only hexane for washing the extracts. Initially, a mixture of dioxins in 60 ml of hexane were put through an 0.5-g Florisil column. After the hexane had drained, the column was eluted with separate 20-ml portions of methylene chloride. All the dioxins were found to be completely retained on the column with no loss in the hexane prewash. All of the dioxins were quantitatively recovered in the first methylene chloride eluate. The same standard mixture was then put through 1.0 g Florisil in 100 ml of hexane. The column was then eluted with four 5-ml portions of methylene chloride, analyzing each portion separately. As seen in Table I, all of the dioxins studied were quantitatively retained on the column even with a 300-ml hexane wash. 2378-TCDD eluted completely in the first 5-ml portion of methylene chloride, however, the higher chlorinated dioxins required at least two additional portions for complete elution. Additional study with standards and sample extracts showed that wash volumes of hexane exceeding 500 ml had no effect on the retained dioxins, while removing fats, oils and other interferents.

Using the original procedure[10], eluates from the acid–silica columns had residues which were not completely soluble in the HPLC injection solvent for the HPSEC system. Even the dioxin fraction from the HPSEC system had residues that were not soluble in the C_8 injection solvent, and even less soluble in the mobile phase. This caused severe problems with the C_8 HPLC system and resulted in very short column life. The Florisil eluates proved to be at least two orders of magnitude cleaner than that of the HPSEC dioxin eluate. This allowed the elimination of the HPSEC system, and greatly improved the performance of the C_8 system with columns lasting a year or more rather than several months.

TABLE I
ELUTION PATTERN OF A STANDARD DIOXIN MIXTURE OFF OF FLORISIL WITH 5-ml PORTIONS OF METHYLENE CHLORIDE FOLLOWING A 300-ml HEXANE PREWASH

Dioxin	*Recovery per 5-ml cut (%)*			
	1	*2*	*3*	*Total*
2378-TCDD	104	–	–	104
124679-PCDD	67	25	7	99
123679-PCDD	74	23	7	104
123678-PCDD	77	18	4	99
123789-PCDD	67	27	3	97
1234679-PCDD	50	40	–	90
1234678-PCDD	70	41	–	111
OCDD	13	57	30	100

Since polychlorinated biphenyl (PCB) residues are frequently found in fish at high levels, the separating power of Florisil was investigated in regard to PCBs. An amount of Arochlor 1254 equivalent to 1 ppm in a fish sample (20 μg) was placed on a 2-g Florisil column and eluted with 200 ml of hexane. More than 99.9% of the PCB was found to wash through the Florisil with the hexane, indicating an excellent separation from the dioxins, and good agreement with the results of Firestone[12].

The separating power of Florisil was further demonstrated by two hazardous waste samples analyzed for 2378-TCDD. These wastes contained up to 200 components including PCBs, polybrominated biphenyls, all types of chlorinated and phosphated insecticides, herbicides, fungicides, phthalates, wood preservatives and other chemicals. These samples were simply diluted with hexane and put through a Florisil column, followed by several hexane rinses. After HPLC cleanup, the GC–ECD chromatograms of the samples were virtually identical to that of the reagent blank. No interfering peaks were found and the recoveries of 1378-TCDD were excellent. No 2378-TCDD was detected.

Due to the high cost of dioxin standards, no attempt was made to determine the adsorptive capacity of Florisil. However, the analysis of high-level samples indicate that the capacity is quite high, and more than adequate for ppt levels. Two river sediment samples exhibited levels of up to 11 ppb for OCDD which represents a loading of 220 ng on the Florisil (2 g) for only the OCDD. In addition, a treated wood sample containing 38 ppb OCDD represented a loading of 140 ng for OCDD alone.

The linearity of the system was tested by analyzing duplicate portions of an egg sample fortified at four different levels, ranging from 13 to 130 ppt for 124679-PCDD to 41 to 410 ppt for OCDD. All the recoveries were essentially complete, and no significant difference in recovery values were found between the four spike levels. In addition, the sediment samples mentioned above, which contained high levels of the higher chlorinated dioxins, gave the same results for the analysis of a 2-g or 20-g sample.

Florisil has proven to be an excellent trap for the dioxins studied as shown by the cleanliness of the extracts, its separating abilities from other contaminants, its adsorptive capacity, and its lincarity. As additional dioxin and furan standards were obtained, they were tested on the Florisil and all were found to behave similarly.

C_8 HPLC

As seen in Fig. 1, this system offers the most selectivity and flexibility in terms of window selection. Any single window, or the window containing all of the dioxins and furans, can be collected. Obviously the smaller the window selected, the cleaner the extract will be. In practice, the TCDD window is usually collected separately if the penta-dioxins are also to be determined, since there is a slight overlap of their respective windows on the C_{18} system. The system is reproducible and standard injections are normally needed only at the beginning and end of the day. Once the elution pattern of all the residues on a particular system has been determined, only individual standards need to be injected to isolate any desired window.

The back-pressure of the pump is a good indicator of system performance. Some extracts may still have some particulate matter present in their solution prior to injection. Multiple injections of these extracts may cause clogging of prefilters or column frits, and these problems are indicated by slight increases in back-pressure. If the back-pressure gets too high, back-flushing of the columns and prefilters with acetonitrile is indicated. As mentioned previously, the columns are routinely cleaned after each sample injection by switching to acetonitrile for about 20 min, and the switching back.

Methylene chloride extraction

Due to the collection of larger volumes of mobile solvent, the original benzene extraction[10] proved to be inconvenient and incomplete. This required the development of another extraction procedure using methylene chloride instead of benzene. Aqueous dilution of the total volume of mobile solvent collected was necessary to minimize the amount of acetonitrile coextracted with the small volumes of methylene chloride used. The total volume of methylene chloride extract was adjusted so as not to exceed the volume of a 10-ml Mills tube. This required a minimum of a 1:4 (v/v) dilution with the bicarbonate solution. For small windows, larger dilutions are recommended to save time in the subsequent evaporation step. Occasionally small crystals of salt are observed on the wall of the tube after evaporation. They do not interfere in the next step.

Initial investigation found that the extraction of 2378-TCDD to be complete in the first 2-ml methylene chloride extract with the remaining extracts serving as rinses. The recovery of 2378-TCDD averaged 99.3% $\pm$ 3.8% for three determinations. The recovery of a mixture of seven higher chlorinated dioxins only through the extraction averaged 100.8%, with a range of 97–111%. A recovery of the same seven dioxins after injection, collection, and extraction averaged 99.4% with a range of 97–104%.

C_{18} HPLC

As seen in Fig. 2 and 3, this system offers lower selectivity in terms of windows for the lower chlorinated dioxins and furans. Its primary advantage is in the complete separation of the hepta- and octa-furans from the other residues. Both OCDD and OCDF have identical GC–ECD retention times, as do at least one hepta-dioxin/furan pair. Therefore, the hepta-/octa-furan window must be excluded from the lower chlorinated window, and collected and determined separately if their analysis is required.

Gas chromatography

The GC–ECD chromatograms of reagent blanks are generally clean in the areas of interest, and the number of extraneous peaks is dependent on the quality of the solvents used. Even with the best of solvents, some peaks were always present, but they seldom interfere with the desired residues. These facts however emphasize the need for taking blanks through the entire procedure. For sample chromatograms, no interference problems were generally encountered for the tetra-, hepta- and octa-congeners. Low-level peaks were occasionally observed for some of the lower congeners; however, their levels were not considered toxicologically significant enough to justify reanalysis for the specific residue. Many of these peaks were eliminated through adjustment of the GC temperature program, or by using the standard coinjection technique.

TCDD validation

Table II is a statistical compilation of data for the recovery of 1378-TCDD from various products. Most of our efforts have concentrated on the analysis of fish and seafood; however, sufficient samples of other products have been analyzed to demonstrate the applicability of this procedure to their analysis. The average recovery of 1378-TCDD from all products studies is adequate, and there is little statistical difference between the various products in terms of recovery and standard deviation. The recovery of 1378-TCDD from reagent blanks has had a tendency to be lower than in samples. Even using other procedures, the presence of a sample matrix has tended to give higher TCDD recoveries presumably due to the presence of residual oils which may act as a "keeper". Since all 2378-TCDD results are corrected for 1378-TCDD recovery, this is not considered a critical point.

TABLE II
RECOVERY OF 1378-TCDD INTERNAL STANDARD FROM VARIOUS PRODUCTS

Product	*Recovery (%)*	
	Average ± S.D.	*n*
Fish	89.5 ± 11.6	126
Milk	90.4 ± 12.4	21
Eggs	92.2 ± 12.0	13
Sediments	93.9 ± 16.2	7
Misc.	94.2 ± 12.1	16
Reagent blanks	86.2 ± 10.7	29

Two fish samples (carp and catfish) containing bio-incurred levels of 2378-TCDD were used as control samples. One portion of a control was routinely analyzed with each batch of samples being analyzed for 2378-TCDD to assure consistent recovery through the procedure. As seen in Table III, the combined results for each species are reproducible and have comparable standard deviations. Also included in Table III are recovery data for various products fortified with 2378-TCDD at a nominal level of 50 ppt. Again,the recoveries are consistent and essentially complete.

TABLE III
2378-TCDD RECOVERIES CORRECTED FOR INTERNAL STANDARD RECOVERY

Sample	*Recovery (%)*	
	Average ± S.D.	*n*
Control samples containing bio-incurred 2378-TCDD		
Carp	84 ± 5.2 ppt	6
Catfish	75 ± 8.7 ppt	8
Spiked samples		
Eggs	94.3 ± 7.6%	12
Fish	94.5 ± 9.3%	6
Milk	97.3 ± 6.3%	4
Gelatin	98.0 ± 14.0%	2
Rice	89.0 ± 11.0%	2
Honey	106.0 ± −%	1
Spiked reagent blanks		
	97.3 ± 8.3%	6

Interlaboratory studies

Table IV is a statistical comparison of analytical data for 1378/2378-TCDD generated in Detroit and Chicago districts. The Chicago laboratory used similar instrumentation and the identical procedure. The close correlation of the results indicate that the procedure is reproducible and rugged between laboratories.

Detroit district also participated in a blind quality assurance study in cooperation with the Michigan Department of Natural Resources (DNR). The DNR took samples of game fish from a Michigan river, composited them, and split the composites between Food and Drug Administration, Detroit and Dow Chemical, Midland, MI for 2378-TCDD analysis. Detroit used the proposed procedure, while Dow used a GC–MS procedure. The results were submitted directly to the DNR for evaluation. Table V is a comparison of the results obtained by both laboratories. The results indicate close agreement between the two procedures.

TABLE IV
INTERLABORATORY COMPARISON OF TCDD RECOVERIES FROM FISH

Lab.	*Recovery (%)*			
	1378-TCDD		*2378-TCDD*	
	Average ± S.D.	*n*	*Average ± S.D.*	*n*
Chicago	92 ± 10	26	102 ± 6	2
Detroit	90 ± 12	126	95 ± 9	6

TABLE V

INTERLABORATORY COMPARISON OF SPLIT-SAMPLE RESULTS FOR 2378-TCDD IN FISH

Blind study prepared by Michigan DNR (see text).

Sample No.	*Species*	*Dow[a] results (ppt)*	*Detroit results (ppt)*
1	Walleye	5.2	2.9
2	Walleye	5.1	3.5[b]
3	Walleye	3.0	3.1
4	Walleye	2.6	1.4
5	N. Pike	15.0	16.5
6	Bass	5.8	8.0
7	Crappie	4.4	5.4
8	W. Bass	15.0	15.9

[a] GC–MS procedure.

[b] 95% Recovery of 2378-TCDD from a separate portion fortified at 50 ppt.

Validation of PCDDs and PCDFs

The validation of these procedures for the higher chlorinated dioxins is based on recovery data of fortified samples. The results for these dioxins are calculated by direct comparison of sample GC–ECD responses to that of a similarly injected standard. The GC retention times these congeners are too far removed from TCDD for internal standard correction. In addition, peak shapes and responses at these long retention times are more dependent on other factors such as matrix effects, and type of injector used.

Initial evaluation of the recovery data for each product indicate that they are statistically similar. In the interest of simplicity, the statistical data for each dioxin includes all of the various products analyzed, and the digestion procedure used. The results are presented in Table VI. As can be seen, the recoveries are consistent and acceptable. Table VII presents the recovery data from spiked reagent blanks taken through the varous extraction digestion procedures. Again good recoveries and precision were indicated, and the results are comparable to the spiked sample data.

TABLE VI

RECOVERY OF DIOXINS AND FURANS FROM ALL SAMPLE TYPES

Most spike levels vary from 10 to 60 ppt in proportion to their GC retention time.

Residue	*Recovery (%)*		*Residue*	*Recovery (%)*	
	Average ± S.D.	*n*		*Average ± S.D.*	*n*
12347-PCDD	100.0 ± 11.6	4	1234678-PCDD	93.9 ± 8.6	34
12378-PCDD	99.7 ± 12.8	6	OCDD	85.0 ± 8.0	29
124679-PCDD	95.0 ± 9.0	30	2378-PCDF	96.2 ± 10.4	5
123679-PCDD	94.3 ± 9.7	34	12378-PCDF	100.0 ± 11.6	5
123478-PCDD	92.1 ± 12.5	7	123478-PCDF	94.4 ± 11.8	5
123678-PCDD	96.3 ± 9.1	32	1234678-PCDF	100.0 ± 10.8	5
123789-PCDD	97.0 ± 9.2	34	OCDF	87.4 ± 15.6	5
1234679-PCDD	92.8 ± 9.6	34			

TABLE VII
RECOVERY OF DIOXINS FROM SPIKED REAGENT BLANKS
Spike levels vary from 10 to 60 ppt in proportion to their increasing GC retention time.

Residue	*Recovery (%)*	
	Average ± S.D.	*n*
124679-PCDD	98.3 ± 5.8	7
123679-PCDD	98.6 ± 4.4	7
123678-PCDD	102.0 ± 7.9	7
123789-PCDD	100.0 ± 8.7	7
1234679-PCDD	96.9 ± 6.5	7
1234678-PCDD	99.9 ± 8.1	7
OCDD	89.0 ± 15.0	7

The validation of these procedures for the furans is also based on spiked sample recovery data. The furan recovery data are presented in Table VI. Although considerably fewer determinations were performed, the furan recovery data closely parallel that of the dioxins.

Results of sample analysis

The bulk of our dioxin work consisted in the analysis of fish for 2378-TCDD, and representative data for these analyses are presented in Table VIII. As can be seen, the highest levels and incidence of TCDD levels occurred in fresh water bottom feeders from lakes and rivers receiving heavy loads of industrial wastes. Fresh water game fish tended to be lower in both incidence and dioxin level. Salt water species exhibited little or no contamination.

Table IX presents the results of market basket survey of commodities analyzed for only the higher chlorinated dioxins. The survey consisted of five commodities sampled from five different geographical areas of the country. The tabulated data includes only the more significant hepta- and octa-dioxin levels since the results for the lower congeners are considered negligible at less than 5 ppt. In general, liver was found

TABLE VIII
INCURRED LEVELS OF 2378-TCDD DETECTED IN FISH AND SEAFOOD
All results confirmed by GC–MS.

Sample type	*No. samples analyzed*	*No. samples positive*	*Average (ppt)*	*Range (ppt)*
Freshwater				
Carp	13	10	31.4	7–90
Catfish	17	16	25.6	5–85
Gamefish	10	4	15.0	13–17
Saltwater				
Fish	55	3	8.0	2–11
Shellfish	18	2	7.0	5–9

TABLE IX
RESULTS OF MARKET BASKET SURVEY
Analyzed for higher chlorinated dioxins only. Results above 20 ppt confirmed by GC–MS, or GC with Hall electrolytic conductivity detection.

Product	*No. samples analyzed*	*1234678-PCDD*			*OCDD*		
		No. samples positive	*Avg. (ppt)*	*Range (ppt)*	*No. positive samples*	*Avg. (ppt)*	*Range (ppt)*
Beef liver	15	12	9.6	3–21	13	47.6	11–182
Pork chops	15	9	6.1	2–20	13	19.8	6–80
Chicken	15	6	8.5	2–32	9	12.4	4–43
Ground beef	15	6	4.0	2–5	4	11.3	6–22
Eggs	15	3	2.7	2–4	3	6.3	5–8

to have the highest levels of dioxins and the highest incidence. Pork, chicken, ground beef and eggs follow in decreasing levels of occurrence. With the limited data, no correlation between dioxin level and geographical area could be made at this time.

All other products or sample types which have shown sigificant levels of dioxins or furans are listed in Tables X and XI. Of interest are the high levels of higher chlorinated dioxins in river sediments located downstream from a paper plant. The sediment samples are also the only samples which contained significant levels of furans. The high levels found in the preservative-treated wood sample, and in the egg sample were the result of specific grower-related problems.

TABLE X
INCURRED LEVELS OF DIOXINS[a] IN OTHER SAMPLE TYPES

Sample type	*No.*	*Dioxin congener (ppt)*					
		124679	*123679*	*123678*	*1234679*	*1234678*	*OCDD*
Eggs		0	0	26	29	127	451
Preserved wood		813	277	269	3800	5600	38 000
Honey skimmings	1	0	0	0	37	63	392
	2	0	0	0	24	37	193
River sediments	1	0	0	0	52	27	172
	2	66	0	0	0	0	65
	3	19	30	42	124	186	270
	4	0	0	0	467	210	1600
	5	61	45	18	870	398	4900
	6	64	41	14	902	393	4600
	7	129	80	26	1600	471	7100
	8	136	55	29	1900	908	10 300
	9	318	379	429	3300	2700	10 600

[a] Only the more significant dioxin levels are included.

TABLE XI
INCURRED LEVEL OF FURANS IN RIVER SEDIMENTS

Sample No.	*Furan congener (ppt)*		
	1234678	*1234689*	*OCDF*
1	15	27	23
5	0	356	348
6	0	395	383

Isomer specificity

Due to the high cost of reliable standards as well as a lack of sources for reliable standards, not all of the isomers for each of the dioxin and furan congeners were included in this study. An attempt was made to include a reasonable cross-section of isomers for each of the congeners, and included as much as possible, those isomers considered to be the most toxic, namely, those having substitution in the 2,3,7,8 positions.

Considering the cross-section of isomers actually studied, it seems reasonable that the remaining isomers would behave similarly, at least quantitatively. Isomer specificity, therefore, is limited to those congeners for which all the isomers were available, namely the 21 isomers of TCDD, the hepta-isomers of the PCDDs and PCDFs, OCDD and OCDF.

Whether or not the chromatographic systems would adequately separate all of the penta- or hexa-isomers does not appear critical. Based on the analytical results, any significant levels of hexa-dioxins were always accompanied by much higher levels of the hepta- and octa-dioxins, thus minimizing the importance of the lower congener. This follows the general trend that, in environmental samples the lower congeners result from the degradation of the higher congeners. In addition, no PCDFs have been detected in any of the foodstuffs analyzed. These statements are made in a general sense, however, and do not preclude the fact that in special cases, isomer specificity could be a problem.

CONCLUSION

Multiresidue procedures have been presented for the screening of PCDDs and PCDFs in a variety of products. Through the incorporation of a Florisil trap, sample extracts are clean enough for determination by capillary GC–ECD, and confirmation by GC–MS. Analytical data from fortified samples, and replicate analysis of samples with incurred residues, indicate that recoveries are reproducible and essentially complete. A comparison of data for 2378-TCDD from two independent laboratories showed good agreement with these results, one laboratory using the same procedure, and the other using a GC–MS procedure.

ACKNOWLEDGEMENT

The author wishes to thank James A. Howell, of Western Michigan University, Kalamazoo, Michigan, for his valuable advice and assistance in the preparation of this paper.

REFERENCES

1 D. G. Patterson, J. S. Holler, C. R. Lapeza, L. R. Alexander, D. F. Groce, R. C. O'Connor, S. J. Smith, J. A. Liddle and L. L. Needham, *Anal. Chem.*, 58 (1986) 705.
2 R. A. Hummel, *J. Agric. Food Chem.*, 25 (1977) 1049.
3 L. A. Shadoff, R. A. Hummel and L. Lamparski, *Bull. Environ. Contam. Toxicol.*, 18 (1977) 478.
4 D. J. Jensen, M. E. Getzendaner, R. A. Hummel and J. Turley, *J. Agric. Food Chem.*, 31 (1983) 118.
5 L. M. Smith, D. L. Stalling and J. L. Johnson, *Anal. Chem.*, 56 (1984) 1830.
6 P. W. Albro, J. S. Schroeder, D. J. Harvan and B. J. Corbett, *J. Chromatogr.*, 312 (1984) 165.
7 L. L. Lamparski, N. H. Mahle and L. A. Shadoff, *J. Agric. Food Chem.*, 26 (1978) 1113.
8 L. L. Lamparski and T. J. Nestrick, *Anal. Chem.*, 52 (1980) 2045.
9 P. W. Albro, W. B. Crummet, A. E. Dupuy, M. L. Gross, M. Hanson, R. L. Harless, F. D. Hileman, D. Hilker, C. Jason, J. L. Johnson, L. L. Lamparski, B. P. Y. Lau, D. D. McDaniel, J. L. Meehan, T. J. Nestrick, M. Nygren, P. O'Keefe, T. L. Peters, C. Rappe, J. J. Ryan, L. M. Smith, D. L. Stalling, N. C. A. Weerashinghe and J. M. Wendling, *Anal. Chem.*, 57 (1985) 2717.
10 R. A. Niemann, W. C. Brumley, D. Firestone and J. A. Sphon, *Anal. Chem.*, 55 (1983) 1497.
11 R. A. Niemann, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 976.
12 D. Firestone, *J. Agric. Food Chem.*, 25 (1977) 1274.
13 L. L. Lamparski, T. J. Nestrick and R. H. Stehl, *Anal. Chem.*, 51 (1979) 1453.
14 M. L. Langhorst and L. A. Shadoff, *Anal. Chem.*, 52 (1980) 2037.
15 J. J. Ryan and J. C. Pilon, *Can. Vet. J.*, 24 (1983) 72.
16 J. J. Ryan, R. Lizotte and W. H. Newsome, *J. Chromatogr.*, 303 (1984) 351.
17 R. Baughman and M. Meselson, *Environ. Health Perspect.*, Exptl. Issue No. 5, (1973) 27.
18 D. Firestone, *J. Assoc. Off. Anal. Chem.*, 60 (1977) 354.