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Characterization of Bovine Urine and Adipose Interlaboratory Performance Evaluation Samples Containing Biologically Incorporated Chlorophenols[1]

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In vivo incorporated bovine urine and adipose reference materials were prepared by oral administration of Lindane, 1,2-dichlorobenzene, 2,4-dichlorophenol, 1,2,3,4-tetrachlorobenzene, and pentachlorophenol to cows. The tissues and fluids obtained were homogenized and analyzed for administered compounds and major metabolites. Virtually all of the 20-g dose of 2,4-dichlorophenol was eliminated in the urine within 24 h following administration, and 70% of the Lindane was metabolized to tri- and tetrachlorophenols and excreted in the urine. The rest of the administered Lindane (25%) was deposited unmetabolized in the adipose. Half of the administered tetrachlorobenzene was deposited in the adipose tissue. The urine samples were found to be reasonably stable with chlorophenol concentrations remaining constant over four freeze/thaw cycles. Bovine urine and adipose reference materials are now available to laboratories to facilitate data comparison or to test analytical procedures for these environmentally important toxicants and their metabolites.

INTRODUCTION

Chlorophenols are of interest because of their inherent toxicity, the presence of dioxin impurities in chlorophenols, and their wide distribution in the environment (Lee and Chau, 1983). Chlorophenols have been disseminated through their direct use as fungicides or bactericides and as breakdown products of a wide variety of pesticides (Cremyln, 1978). Despite extensive analysis and study, no single method has emerged as a standard analytical technique for determining chlorophenols in biological tissues and fluids. Some of the techniques that have been used for the analysis of chlorophenols include: GC/ECD determination of underivatized chlorophenols (Kalman, 1984), GC/ECD determination of their derivatives (Edgerton, 1981), GC/MS (Hargesheimer and Coutts, 1983), HPLC of underivatized chlorophenols with electrochemical (McMurtrey et al., 1984) or UV detection (Pelsari and Aitio, 1982), and HPLC of derivatives with fluorescence detection (Carlson et al., 1984). As there are no definitive studies of the accuracy of these various methods, meaningful comparison of analytical results reported in the chemical literature is a difficult proposition.

In addition to the data comparability problems caused by using different analytical methods, chlorophenol analyses are complicated by the fact that these compounds

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Table I. Mixtures of Chlorinated Compounds Administered to Cows

feeding gp	compds administered	daily dose, g
gp A	Lindane	4.0
gp B	Lindane	2.0
	1,2-dichlorobenzene	20.0
	2,4-dichlorophenol	20.0
	1,2,3,4-tetrachlorobnzene	2.0
gp C	Lindane	1.0
	1,2-dichlorobenzene	20.0
	2,4-dichlorophenol	20.0
	1,2,3,4-tetrachlorobenzene	2.0
	pentachlorophenol	1.0

can degrade during storage and analysis. It has been our experience as well as that of others (Edgerton, 1981) that the concentration of chlorophenols in biological matrices decreases significantly over a period of days to weeks. Edgerton also cautions that chlorophenols are lost during analysis unless the sample is protected from light and an antioxidant is added during hydrolysis.

In order to reduce the difficulties in comparing data generated by using different methods of sample preparation and analyses, the EPA's Environmental Monitoring Systems Laboratory in Las Vegas (EMSL-LV) can provide performance evaluation samples containing biologically incorporated chlorophenols in bovine urine and adipose. These serve the purpose of facilitating comparison of results between different laboratories using different analytical methods by allowing the analysis of equivalent samples. We have included our methods of analysis (GC/ECD techniques modified from the *EPA Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples*, 1980) with the results of the analyses in this paper. The kinds of QA materials that can be made available upon request are described in the Conclusion.

EXPERIMENTAL SECTION

Chemicals. Most of the chlorophenols [2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorophenol; 2,4,6-trichlorophenol; 2,3,4,6- and 2,3,4,5-tetrachlorophenol; and pentachlorophenol (PCP)] used as analytical standards were obtained from the EPA Pesticide Repository, Research Triangle Park, NC. All others were obtained from Aldrich Chemical Co. Milwaukee, WI. The chemicals administered to the cows (Lindane, 1,2-dichlorobenzene, 1,2,3,4-tetrachlorobenzene, 2,4-dichlorophenol, PCP) were obtained from the macro-repository maintained by the EPA in North Carolina. Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) was purchased from Aldrich (see caution below). All solvents used were HPLC or distilled in glass grade, and other chemicals were reagent grade from standard sources.

Acquisition of Bovine Tissue and Fluid with Incorporated Chlorophenols. Portions of EPA programs in pesticides, toxics, and hazardous waste have need for biological reference material. The dairy cow was chosen as a source for those materials because of the size of the animal and the volumes of tissues and fluids that could be produced. The cows used in this study were treated with one of three mixtures of chlorophenols, chlorobenzenes, and/or Lindane which are given in Table I. The cocktail of compounds was prepared at the laboratory facilities of the University of Nevada at Reno (UNR) and analyzed there before they were administered in a gelatin capsule using a balling gun. The animals were maintained at the Carey Animal Facility at UNR; prior to administration of the mixtures of chlorinated compounds, the animals were acclimated for 1 week in individual stalls. Samples of urine, serum, and milk were taken during this acclimation period for use as controls. After administration of the compounds began, the collection of urine, serum, and milk samples continued at regular intervals during the 28-day treatment period. Urine was collected with an in-dwelling, inflatable catheter; approximately 19 L of urine/day per cow was collected. Serum samples were collected from the jugular vein, and no more than 250 mL/day or 750 mL/week of blood was collected. Milk samples were collected by a mechanical milking machine. The cream and milk were allowed to separate before storage, and only the cream was frozen in order to ensure the performance evaluation samples were homogeneous after thawing. In addition, approximately 2-kg kidney fat samples and 1-L serum samples were taken at sacrifice.

All samples were transferred to Teflon screw-capped vessels and stored at −20 °C at UNR until they were shipped overnight on dry ice to EMSL-LV. In the Las Vegas EPA Laboratory, the samples were thawed and subsampled into sample bottles or ampules, sealed, and wrapped in aluminum foil before refreezing at −22 °C. Throughout the procedures, care was taken in the preparation of the containers and caps used to hold the sample and the manner in which the sample is taken. Paper-lined caps were used only if a layer of aluminum foil or Teflon was inserted to isolate the sample from the paper liner because paper and wood products frequently contain pentachlorophenol. In this study, no containers or surfaces were used except stainless steel, glass, aluminum foil, or Teflon.

Hydrolysis and Extraction of Urine. Before starting analyses, all glassware was carefully washed to remove the chlorophenol contaminants; all of the tubes used for hydrolysis were also checked for cracks.

A volume of 2.00 mL of thawed urine was transferred to a foil-wrapped 25 × 125 mm culture tube with a Teflon-lined screw cap (wrapping with foil minimizes the photodecomposition of chlorophenols). A volume of 0.5 mL of concentrated hydrochloric acid and 100 mg of sodium bisulfite (an antioxidant) were added. The tube was sealed and the urine hydrolyzed in a boiling-water bath for 1 h.

After the hydrolysis was completed, the tubes were cooled, an additional 100-mg portion of bisulfite was added to each, and the solids were spun down in a table top centrifuge. The supernatant was decanted into a 15-mL foil-wrapped centrifuge tube, and the solids were washed sequentially with 2 mL of water and two 2-mL portions of toluene. The supernatants were combined and mixed for 20 min on a mechanical rotator (30–50 rpm). The phases were separated by centrifugation, and each organic layer was transferred to an amber volumetric flask. Each aqueous fraction was extracted with two additional 2-mL portions of toluene, and all of the extracts were combined. It is critical that the extraction and the methylation procedures be carried out on the same day in order to maximize the recovery of chlorophenols.

Extraction of Adipose. Adipose samples were analyzed by the micro method for chlorinated hydrocarbons presented in the *EPA Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples* (1980). A 0.5-g sample of fat, weighed to the nearest 0.1 mg, was transferred to a foil-wrapped tapered glass tissue grinder with 2.5 mL of acetonitrile. The extraction was accomplished with hand grinding for 1 min. The acetonitrile was transferred to a foil-wrapped centrifuge tube, the solids were spun down, and the solvent was transferred to a foil-wrapped 50-mL centrifuge tube. The acetonitrile extraction was repeated two additional

Table II. Retention Times of Methylated Chlorophenols and Chlorobenzenes Relative to Lindane on Three Columns

compd	column: OV-210, 135 °C isothermal	column: ultrabond PEGS, 135 °C isothermal	column: DB-5 programmed 70 to 240 °C	ECD resp to Lindane on OV-210
1,2-dichlorobenzene	0.44	0.02	nd[a]	2.23
2,3-dichlorophenol	0.15	0.07	0.29	1.48
2,4-dichlorophenol	0.13	0.05	0.25	1.32
3.4-dichlorophenol	0.12	0.05	0.26	0.93
3,4-dichlorothiophenol	0.22	0.11	0.50	2.39
2,3,5-trichlorophenol	0.23	0.11	0.46	3.52
2,4,5-trichlorophenol	0.23	0.11	0.46	4.06
2,4,6-trichlorophenol	0.12	0.04	0.30	10.4
1,2,3,4-tetrachlorobenzene	0.14	0.06	0.36	8.81
2,3,4,5-tetrachlorophenol	0.59	0.33	0.82	2.63
2,3,4,6-tetrachlorophenol	0.28	0.10	0.58	7.29
tetrachlorohydroquinone	0.79	0.29	0.39	2.32
tetrachloropyrocatechol	0.69	0.27	0.91	3.81
pentachlorophenol	0.63	0.23	0.93	3.99
pentachlorothiophenol	1.38	0.73	1.16	5.28
Lindane	1.0	1.0	1.0	1.00

[a] Not observed because of the 30-s value closure after the splitless injection.

times, and 25 mL of 2% aqueous sodium sulfate was combined with the combined acetonitrile fractions on a Vortex mixer.

The aqueous acetonitrile extract was extracted three times with hexane (5, 2, and 2 mL). The hexane extracts were combined in an amber flask, and the volume was reduced to 4–6 mL under a stream of dry nitrogen at 25 °C.

Methylation of Chlorophenols. Methylation of chlorophenols was found to greatly improve their stability and chromatographic properties (Edgerton et al., 1979). Methylation was accomplished by reaction of the chlorophenols with ethereal diazomethane generated in a Mini Diazald distillation apparatus (Aldrich Chemical) according to the manufacturer's instructions. As diazomethane poses a hazard as both a carcinogen and as an explosive, laboratories using this reagent must perform all derivatization in a fume hood and use no ground-glass apparatus in the preparation of the reagent. We recommend that diazomethane be generated as required rather than storing bulk diazomethane in a freezer.

The safest technique for generating diazomethane is to use Diazald and the Clear-Seal distillation apparatus sold by Aldrich Chemical. Following the manufacturer's instructions, a solution containing 0.5–1 g of Diazald in ether was added to aqueous, ethanolic potassium hydroxide in a reaction vessel heated to 70 °C. The ethereal solution of diazomethane was distilled into a receiving flask until the distillate had no color. The receiving flask was removed from the apparatus, and 2-mL aliquots of the diazomethane solution were added to each of the flasks containing chlorophenols. The flasks were stoppered with Teflon stoppers or foil-wrapped corks, swirled, and allowed to stand overnight in the hood. The following day any residual diazomethane was removed and the solvent exchanged to hexane under a stream of dry nitrogen.

Chlorophenols from urine or adipose tissue samples were derivatized not more than 4 h after extraction. After derivatization and solvent exchange, the volume of extract was adjusted to 0.5 mL before alumina column cleanup. Analytical amounts of chlorophenol standards were methylated along with the extracted samples each time a derivatization was performed. The derivatized standards were used to quantitate the chlorophenols in the extracts and to monitor the performance of the derivatization procedure.

Column Cleanup. A portion of acidic alumina, column chromatographic grade 80–200 mesh (J. T. Baker), was transferred to a beaker, loosely covered with aluminum foil, and activated at 110 °C overnight. The following day, cleanup columns were dry packed by loosely plugging a clean 22 × 9 mm chromaflex column (Kontes) with silanized glass wool and adding 4.0 g of alumina followed with 1.6 g of anhydrous sodium sulfate. The columns were then washed with 10 mL of ether followed by 10 mL of hexane before extracts were put on the columns.

After a column was prepared for each methylated extract, the extract was transferred onto the column and the column was eluted with 10 mL of hexane/ether (40:60). The entire eluate was collected and the final volume adjusted to 10.00 mL in a volumetric flask. (*Note*: Ether was substituted for benzene which was specified in the original EPA procedure because benzene is a human carcinogen.

GC Analysis. The GC analyses in these experiments were carried out on two packed (1.8 m × 2 mm) 5% OV-210 on 80–100 mesh Gas-Chrom Q or 1% Ultrabond-PEGS 100–200 mesh (RFR Corp.) and a DB-5 capillary columns (30 m × 0.25 mm with an 0.25-μm coating thickness). The packed-column analyses were performed on a Tracor 222 with a column oven temperature of 135 °C and injector at 200 °C using a pulsed linearized electron capture detector operated at 325 °C. The carrier gas was 10% methane in argon (P-10). The capillary column chromatography was accomplished with a Tracor 540 with a pulsed linearized ECD operated at 300 °C using a splitless injector at 220 °C with a 30-s valve closure. The carrier gas was helium, and the detector makeup was P-10. After an initial 1-min hold, a two-step oven temperature program ran from 70 to 120 °C at 5 °C/min and then from 120 to 240 °C at 20 °C/min with a final 15-min hold. The output of both instruments was integrated with an IBM CS 9000 lab data station.

RESULTS AND DISCUSSION

Chromatography. One of the first tasks in conducting this study was to accomplish a separation of the methylated chlorophenols by GC. For the urine analyses, only the packed-column instrument with two columns was available (PEGS, OV-210). For the adipose analysis, a GC with a DB-5 capillary column was used. Using the capillary column, near base-line resolution of all of the analytes was possible except for the 2,4-, 3,4-dichlorophenol pair; there was coelution of at least five peaks in either of the packed columns. The retention times of the various methylated chlorophenols and chlorobenzenes relative to Lindane on

Table III. Recoveries of Chlorinated Compounds Spiked into Control Urine

compd	spike level, % 1 ppm	10 ppm
2,3-dichlorophenol	81	73
2,4-dichlorophenol	82	81
3,4-dichlorophenol	100	101
3,4-dichlorothiophenol	6	24
2,3,5-trichlorophenol	78	76
2,4,5-trichlorophenol	92	94
2,4,6-trichlorophenol	68	79
2,3,4,5-tetrachlorophenol	87	89
2,3,4,6-tetrachlorophenol	82	79
tetrachlorohydroquinone	38	40
tetrachloropyrocatechol	47	36
pentachlorophenol	96	97
pentachlorothiophenol	84	109
Lindane	61	76
1,2-dichlorobenzene	55	97
1,2,3,4-tetrachlorobenzene	52	53

the three columns used are reported in Table II. A more complete treatment of the behavior of the methyl derivatives of chlorophenols on several other capillary columns is described by Korhonen (1984).

It is our recommendation that a capillary GC be used for the analysis of chlorophenols if it is available. If the analysis is to be done on packed-column GC, the resolution of the chlorophenols was generally better on the more polar column (PEGS), but the peaks from the less volatile compounds were broadened excessively for good quantitation. It appears that at least two packed columns will be required for the separation of the various chlorophenols encountered in these samples.

Electron-capture detection (ECD) was used for analysis of chlorophenols. Because this detector has different sensitivity for each of the analytes, it was necessary to generate a calibration curve for each compound. The response of the packed-column Tracor ECD was plotted for five different concentrations over a 2-order-of-magnitude range. Linear regression analysis was used to generate the best fit line from the data on a Hewlett-Packard 33C calculator. The responses of the different chlorophenols relative to that of Lindane on the OV-210 column are reported in Table II; the response of the detector was linear to >50 ng of chlorophenol/injection with this column and detector.

The results presented in Table II show that ECD response is generally positively correlated with the number of chlorines in the molecule, but specific substitution patterns can have a major influence. For example, 2,4,6-trichlorophenol gives a much higher response than the other trichlorophenols tested and 2,3,4,6-tetrachlorophenol gave almost 3 times the response of 2,3,4,5-tetrachlorophenol.

Recoveries of Chlorophenols from Urine. The recoveries of chlorophenol standards spiked into control urine were used as a model for the recovery of conjugates from the urine of animals administered the compounds listed in Table I. The use of the free phenols was necessary because no conjugated phenols were available to this laboratory. It is expected that the free phenols are an adequate model for the behavior of the conjugates, and it has been previously demonstrated that the hydrolysis procedure used here is sufficient to cleave conjugated phenols in urine (Edgerton et al., 1979). The recoveries from this method are reported in Table III. They range between 68 and 100% for spikes at the 1 ppm level and 73 and 101% for spikes at the 10 ppm level. This study demonstrated that this technique is suitable for the quantitation of chlorophenols in urine. Lower recoveries were observed for the chlorothiophenols, which undergo chemical degradation during the workup (Allsup and Walsh, 1982), and for 1,2-dichlorobenzene, which was the most volatile compound analyzed.

Table IV. Analysis of Urine in PEGS and OV-210 Columns

compd	PEGS column concn, μg/mL	% av, dev	level in control as % of treated B
1,2-dichlorobenzene	9.7	11	nd[d]
2,3-dichlorophenol	25.2	3.9	nd
2,4-dichlorophenol	1084	12	< 0.01%
3,4-dichlorophenol	a		
3,4-dichlorothiophenol	b		
2,3,5-trichlorophenol	b		
2,4,5-trichlorophenol	2.9	4.2	nd
2,4,6-trichlorophenol	2.8	2.1	nd
1,2,3,4-tetrachlorobenzene	a		
2,3,4,5-tetrachlorophenol	38.0	14	< 0.01%
2,3,4,6-tetrachlorophenol	11.8	17	< 0.01%
tetrachlorohydroquinone	0.3	18	nd
tetrachloropyrocatechol	0.7	9.8	nd
pentachlorophenol	<0.1	14	70%
pentachlorothiophenol	1.6	54	nd
Lindane	<0.3	nd	nd
1,2-dichlorobenzene	12.3	17	nd
2,3-dichlorophenol	25.0	5.2	nd
2,4-dichlorophenol	928	6.1	<0.01%
3,4-dichlorophenol	a		
3,4-dichlorothiophenol	<0.15		
2,3,5-trichlorophenol	b		
2,4,5-trichlorophenol	4.3	2.3	nd
2,4,6-trichlorophenol	a		nd
1,2,3,4-tetrachlorobenzene	a		
2,3,4,5-tetrachlorophenol	35.7	16	<0.1%
2,3,4,6-tetrachlorophenol	13.3	15	<0.1%
tetrachlorohydroquinone	<0.1		nd
tetrachloropyrocatechol	1.7	7.9	nd
pentachlorophenol	c		70%
pentachlorothiophenol	<1		nd
Lindane	0.50	5.2	nd

[a] Cochromatographed with 2,4-dichlorophenol. The peak was >95% 2,4-dichlorophenol by capillary GC analysis. [b] Cochromatographed with 2,4,5-trichlorophenol. The peak was >80% 2,4,5-trichlorophenol and 20% 2,3,5-trichlorophenol by capillary GC analysis. [c] Cochromatographed with 2,3,4,5-tetrachlorophenol. [d] nd = not detected.

Chlorophenols in Urine. The levels of chlorophenols found in the urine of a cow from group B collected on day 4 of the feeding study are reported in Table IV. The analyte present in the highest concentration, 2,4-dichlorophenol, was found to be present at a concentration of 1 mg/mL of urine. Although it was not a purpose of this study to provide a material balance of the administered compounds, it appears that almost all of the daily administration of 20 g of 2,4-dichlorophenol was excreted in urine as the average urine production of each cow was 19 L. The hypothesis that virtually all of the administered 2,4-dichlorophenol was excreted via the urine is supported by the fact that no dichlorophenol was detected in the fat. Much lower levels of 2,3-dichlorophenol (25 μg/mL) and 3,4-dichlorophenol were also detected in the urine. These are presumed to be metabolites of 1,2-dichlorobenzene and of Lindane (Karapally et al., 1973) or impurities in the administered 2,4-dichlorophenol.

Tetrachlorophenols were found at the next highest concentration in the urine. Both of the observed tetrachlorophenols (2,3,4,5 and 2,3,4,6) have been reported as Lindane metabolites (Freal and Chadwick, 1973). In addition, 2,3,4,5-tetrachlorophenol could result from the oxidative metabolism of 1,2,3,4-tetrachlorobenzene. The

three trichlorophenols detected in the urine (2,3,5; 2,4,5; 2,4,6) have been demonstrated to be Lindane metabolites (Freal and Chadwick, 1973). On the basis of a calculation using the total molar concentration of tri- and tetrachlorophenols in the urine multiplied by a total volume of 19 L of urine, 70% of the administered Lindane is excreted as tetra- and trichlorophenols. This observation is supported by observations in rats (Freal and Chadwick, 1973) and in rabbits (Karapally et al., 1973).

Only a small amount (<1%) of the administered 1,2-dichlorobenzene was observed in the urine. No 1,2,3,4-tetrachlorobenzene could be detected on either packed column because it coelutes with 2,4-dichlorophenol. Subsequent capillary GC analyses demonstrated that no detectable levels (>0.05 μg/mL) of tetrachlorobenzene were excreted in the urine. Relatively large amounts of both of these apolar compounds were present in adipose.

Pentachlorophenol was also present in the urine of all of the animals used in this study even if they were not administered PCP, demonstrating the ubiquitous nature of chlorophenol contamination in the environment.

The precision of this method is demonstrated by the range of values reported for each compound determined. The relative confidence interval for all chlorophenols using this method was calculated as 9% by the relationship confidence interval = $t\sigma/n^{1/2}$, where n = number of replicate analyses, t = value from the Student's t-test, and σ = largest relative standard deviation.

These values indicate that this technique for analyzing chlorophenols is adequate for our purposes. Some of the factors that contributed to the lack of precision were the chemical reactivity of the chlorophenols and their photochemical instability, as well as the normal problems of quantitation in residue analysis associated with hydrolyzing, extracting, and derivatizing conjugates in biological media. It is our belief that the problems of photochemical degradation and air oxidation during hydrolysis were the greatest causes of variability in these chlorophenol analyses. While there are measures included in this method to reduce these problems (i.e., wrapping glassware with foil and adding bisulfite), it is very possible that some of the alternate methods such as HPLC or a recently published GC method, using a chloroacetate derivatization of chlorophenols in water (Lee et al., 1985) could give more precise results.

For samples to be useful as interlaboratory comparison samples, they must be stable during storage and shipment. Edgerton (1981) has reported that chlorophenols excreted in urine are sensitive to freezing and thawing over a period of weeks. Therefore, an investigation was conducted to determine whether these samples could survive being thawed and refrozen over a short period of time as might occur during delivery to another laboratory. Several ampules were thawed and refrozen four times over a 2-day period and then reanalyzed. The results are reported in Table V. Those data indicate that no significant breakdown of the analytes occurred; thus, the concentration of chlorophenols in these urine samples should not change during shipment of interlab samples. A study of the storage stability of organic toxicants in cow tissue has been initiated at S-CUBED, La Jolla, CA. The results of this study will be used to determine how long samples can be stored before it is necessary to reestablish the concentration of toxicants in tissue PE samples.

Fat Samples. A second set of bovine interlab samples available from this project are from cows of feeding group C. A representative kidney fat sample taken at sacrifice was analyzed, and the levels of chlorophenols were determined by the micro procedure of Mills, Olney, and Gaither (EPA, 1980) described above in the Experimental Section. No attempt was made to liberate conjugated phenols from adipose because it was expected that only phenols should have partitioned into adipose tissue.

Table V. Analysis of Urine before and after Four Freeze/Thaw Cycles (Concentrations, μg/mL)

	PEGS column	
compd	thawed once	frozen/ refrozen 4×
1,2-dichlorobenzene	9.7	9.1
2,3-dichlorophenol	25.2	22.0
2,4-dichlorophenol	1071	706
3,4-dichlorophenol		[a]
3,4-dichlorothiophenol		[b]
2,3,5-trichlorophenol		[b]
2,4,5-trichlorophenol	2.9	2.5
2,4,6-trichlorophenol	2.8	2.0
1,2,3,4-tetrachlorobenzene		[a]
2,3,4,5-tetrachlorophenol	38.0	40.3
2,3,4,6-tetrachlorophenol	11.8	10.5
tetrachlorohydroquinone	0.3	<0.1
tetrachloropyrocatechol	0.7	0.7
pentachlorophenol	0.1	<0.1
pentachlorothiophenol	1.6	3.9
Lindane	<0.3	<0.3
	OV-210 column	
compd	thawed once	frozen/ refrozen 4×
1,2-dichlorobenzene	12.3	6.9
2,3-dichlorophenol	25.0	18.6
2,4-dichlorophenol	928	876
3,4-dichlorophenol	[a]	[a]
3,4-dichlorothiophenol	<0.15	<0.15
2,3,5-trichlorophenol	[b]	[b]
2,4,5-trichlorophenol	4.3	3.5
2,4,6-trichlorophenol	[a]	[a]
1,2,3,4-tetrachlorobenzene	[a]	[a]
2,3,4,5-tetrachlorophenol	35.7	41.3
2,3,4,6-tetrachlorophenol	13.3	15.7
tetrachlorohydroquinone	<0.1	<0.1
tetrachloropyrocatechol	1.7	1.0
pentachlorophenol	[c]	[c]
pentachlorothiophenol	<1	<1
Lindane	0.50	0.36

[a] Cochromatographed with 2,4-dichlorophenol. [b] Cochromatographed with 2,4,5-trichlorophenol. [c] Cochromatographed with 2,3,4,5-tetrachlorophenol.

Table VI. Concentration of Chlorinated Toxicants in Adipose Reference Standards

compd	concn in adipose, μg/g
1,2-dichlorobenzene	258
2,3-dichlorophenol	nd
2,4-dichlorophenol	nd
1,2,3,4-tetrachlorobenzene	392
2,3,4,5-tetrachlorophenol	2.5
2,3,4,6-tetrachlorophenol	2.4
pentachlorophenol	0.93
Lindane	91.0

The levels of the chlorinated species detected by capillary GC in fat are reported in Table VI. Because of the complexity of the adipose chromatograms, only "high-level" analytes were determined. The chlorophenols present at greater than 1 μg/g were 2,3,4,6- (2.8 μg/g) and 2,3,4,5-tetrachlorophenol (2.1 μg/g). Those concentrations were less than are observed in urine. A concentration of 0.9 μg/g of PCP was also detected. As expected, no di- or trichlorophenols were observed at this sensitivity, because they are water soluble and were excreted via the urine (Table IV). The fat sample also contained significant amounts of unmetabolized 1,2-dichlorobenzene (258 μg/g),

1,2,3,4-tetrachlorobenzene (392 μg/g), and Lindane (91 μg/g). On the basis of the concentration of these analytes in adipose multiplied by the 80 kg of adipose tissue in the cow (approximately 15% of the body weight [550 kg] was fat), more than half of the administered tetrachlorobenzene, 25% of the Lindane, and less than 5% of the dichlorobenzene are deposited in the adipose of the animals.

Deposition and Excretion of Administered Compounds. Urine samples from a cow of feeding group B (Table I) and adipose samples from a cow of group C were analyzed as part of this study. The results given above in the urine and adipose sections (Tables IV and VI) can be combined to give information of the fate of the administered compounds. These data indicate that virtually all of a 20-g administration of 2,4-dichlorophenol is eliminated via the urine within the 24 h following administration. Roughly 70% of a daily administration of Lindane is metabolized to more polar tetrachloro- and trichlorophenols then eliminated via the urine. Most of the remaining Lindane (25% of that administered) was deposited in the bovine adipose. More than 50% of the administered tetrachlorobenzene was present in the adipose at sacrifice, and none was detected in the urine. The milk samples remain to be analyzed and very probably contain some tetrachlorobenzene. Less than 5% of the administered dichlorobenzene was recovered in the adipose and only 1% was eliminated in the urine. Much of the remainder of the dose was probably volatilized out of the stomach of the cow because of the nature of ruminant physiology. A similar calculation of the fate of PCP cannot be made because of the relatively high background levels of PCP and because PCP was only fed to animals of group C (urine from that group has not been analyzed). The adipose data we have demonstrates that, even at high dose levels, only a small amount (<3%) of the administered PCP is deposited in fat.

CONCLUSION

The measurement of organic toxicants in biological media is an important aspect of environmental monitoring, but monitoring activities are not just limited to the measurement of toxicants in individuals. The measurements must be incorporated into a data base so that the larger picture emerges. A number of groups are engaged in the measurement of chlorophenol residues in animals and they have developed a variety of different analytical procedures. This has led to difficulties in incorporating the results into a common data base. This problem of interlaboratory comparison is further complicated by the fact that animals exposed to chlorophenols metabolize them to chemical species different from those dispersed into the environment. For this reason, spiked control tissue is not a good laboratory intercomparison sample. In order to have quality assurance of the analytical measurement of environmental chlorophenols, it is necessary to have refernce materials that contain their in vivo transformation products so that the different analytical methods can be compared.

A cooperative venture between the USEPA's Environmental Monitoring Systems Laboratory in Las Vegas (EMSL-LV) and the University of Nevada at Reno has produced relatively large volumes of tissues and fluids that contain biologically incorporated chlorophenols as well as other chlorinated toxicants. The urine and adipose samples described in this paper are currently available from EMSL-LV as laboratory evaluation samples.

The analysis of serum and milk from the same study is under way at the University of Nevada at Reno, and those samples should be available in the future. It is the hope of this group that different laboratories will use these samples in order to test their analytical methods for chlorophenols on actual biological samples.

We plan to continue this work and make a wider variety of materials available as reference materials. This year another group of cows has been given a cocktail containing a wider variety of organic toxicants including hexachlorobenzene, Aroclor 1254, polybrominated biphenyl, PCP, Carbaryl, dioctyl phthalate, methylenedianiline, and yellow OB (an azo dye). Work is in progress at EMSL-LV to characterize those samples so that they can be made available in the near future.

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Registry No. PCP, 87-86-5; o-$Cl_2C_6H_4$, 95-50-1; Lindane, 58-89-9; 2,4-dichlorophenol, 120-83-2; 1,2,3,4-tetrachlorobenzene, 634-66-2; 2,3-dichlorophenol, 576-24-9; 3,4-dichlorophenol, 95-77-2; 3,4-dichlorothiophenol, 5858-17-3; 2,3,5-trichlorophenol, 933-78-8; 2,4,5-trichlorophenol, 95-95-4; 2,4,6-trichlorophenol, 88-06-2; 2,3,4,5-tetrachlorophenol, 4901-51-3; 2,3,4,6-tetrachlorophenol, 58-90-2; tetrachlorohydroquinone, 87-87-6; tetrachloropyrocatechol, 1198-55-6; pentachlorothiophenol, 133-49-3.

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