Procedure for the Determination of Residues of (2,4-Dichlorophenoxy)acetic Acid in Dermal Exposure Pads, Hand Rinses, Urine, and Perspiration from Agricultural Workers Exposed to the Herbicide

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Residues of (2,4-dichlorophenoxy)acetic acid (2,4-D), its isooctyl or butoxyethanol esters, and its dimethylamine salt were determined in dermal exposure pads, hand rinses, urine, and perspiration obtained from workers involved in the application of the herbicide. The 2,4-D esters or dimethylamine salt residues were extracted from dermal exposure pads with methyl alcohol and directly converted to the methyl ester with boron trifluoride. Residues of 2,4-D in perspiration samples were purified with a reverse-phase liquid chromatographic support (octadecyl) and converted to the methyl ester. Residues of 2,4-D, its esters, or its dimethylamine salt in hand rinses and urine were treated with potassium hydroxide, purified with a reverse-phase liquid chromatographic support, and converted to the methyl ester. The derivatized residues were quantitated by electron capture gas chromatography and satisfactory recoveries were obtained at a detection limit of 0.02 μg of 2,4-D/cm^2 for dermal exposure pads, 0.03 μg/mL for hand-rinse samples, 0.07 μg/mL of urine, and 1.0 μg/T-shirt for each perspiration sample.

The broad-spectrum herbicide (2,4-dichlorophenoxy)-acetic acid (2,4-D) is widely used to control broad-leaved weeds. Because of concern for the safety of agricultural workers handling pesticides, a study was initiated to determine exposure to 2,4-D. Agricultural workers involved in the application of 2,4-D were monitored for dermal exposure rates and levels of the herbicide they excreted through urine and perspiration. Since this study involved a large number of samples, a rapid, sensitive, and accurate analytical method that would be applicable to 2,4-D, its isooctyl or butoxyethanol esters, and its dimethylamine salt was desired.

Glas (1978) reported a method for determining 2,4-D in human urine. Other methods have been reported for determining 2,4-D and other phenoxy herbicides in soil (Olson et al., 1978), in water (Agemian and Chau, 1977; Devine and Zweig, 1969), and in animal blood, urine, and tissue (Clark, 1969). These procedures use solvent partitioning alone, or in combination with column chromatography for sample cleanup, and would be too time consuming for our purposes.

Also, 2,4-D is usually esterified or transesterified to an alkyl ester for gas chromatographic analysis. The 2-chloroethyl (Chau and Terry, 1975), 2,2,2-trichloroethyl (Mierzwa and Witek, 1977) and pentafluorobenzyl (Agemian and Chau, 1977) esters of 2,4-D are highly sensitive to electron capture detection but are time consuming to prepare. Horner et al. (1974) showed that esterifying 2,4-D with boron trichloride in methyl alcohol gave yields of greater than 90% in less than 20 min and did not produce the impurities that diazoalkylation produced. Pursley and Schall (1965) use a solution of boron trichloride in methyl alcohol to quantitiatively transesterify various esters of 2,4-D in commercial formulations to the methyl ester.

This paper described a procedure based on a modification of the work of Pursley and Schall (1965) in which BF_3 in methyl alcohol is used to directly convert residues of 2,4-D, its esters, and its amine salt to the methyl ester prior to gas chromatographic analysis. A rapid cleanup of residues of 2,4-D, 2,4-D ester, or 2,4-D dimethylamine salt was obtained with a commercially available cartridge of reverse-phase liquid chromatographic support. The esters of 2,4-D were hydrolyzed with KOH to 2,4-D before using this cleanup technique.

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EXPERIMENTAL SECTION

Chemicals. The solvents, methyl alcohol (MeOH), dichloromethane ($MeCl_2$) and 95% ethyl alcohol (EtOH) were technical grades redistilled in glass. The hexane was chromatographic grade and the water was purified by reverse osmosis (Culligan Co., Northbrook, IL). Anhydrous sodium sulfate (Na_2SO_4), potassium hydroxide (KOH), and phosphoric acid (H_3PO_4) were reagent grade. Acidified water contained 0.2% H_3PO_4. The boron trifluoride (BF_3) reagent was a commercially available mixture of 14% BF_3 in MeOH (Pierce Chemical Co., Rockford, IL). Standard solutions of 2,4-D, its isooctyl ester, its butoxyethanol ester, and its dimethylamine salt were prepared by dissolving 0.1000 g of the pure compounds in 500 mL of either $MeCl_2$ or MeOH. These stock standard solutions were diluted as desired. Portions of 2,4-D, its esters, and dimethylamine salt standards were methylated by the procedure described in this paper and used as standards for the gas chromatographic analysis of samples. The purity of these standards were compared with a commercially available 2,4-D methyl ester standard (99.5% purity, Nanogen Co., Watsonville, CA), and were found to be of equal quality. Also, a standard solution of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) was prepared in the same manner. Dilutions of this standard were added to samples as an identifier (not an internal standard) to determine if any qualitative errors were made in the analysis of samples.

Equipment. The octadecyl reverse-phase liquid chromatographic cartridges (Sep-PAK, Waters Associates, Milford, MA) were preconditioned on the day of their use by flushing with 2.0 mL of MeOH followed by 5.0 mL of water. The cartridges that could be reused were regenerated by reverse flushing successively with 10 mL each of $MeCl_2$, ETOH, MeOH, and water.

The gas chromatograph (GLC) was a Hewlett-Packard Model 5840A, equipped with an electron capture detector. The GLC column was a 122 cm × 4.0 mm i.d. glass column packed with 3% silar 5CP on Gas-Chrom Q (100–120 mesh). The column oven was operated isothermally at 200 °C, and the injector and detector temperatures were 225 and 300 °C, respectively. The carrier gas was a mixture of 95% argon and 5% methane, and the flow rate was 50 mL/min.

Analysis of Dermal Exposure Pads. Dermal exposure samples were taken by a modification of Durham and Wolfe's procedure (1962). In the modified procedure, absorbent gauze pads were attached to disposable jackets worn by workers when mixing, loading, and applying 2,4-D

formulations. The pad construction and field sampling techniques are described by Maitlen et al. (1982).

After use, each exposure pad was trimmed to 42.3 cm^2, folded, and placed in a 100-mL widemouth bottle containing 50 mL of MeOH. In the laboratory the bottle was opened, and 0.5 μg of 2,4,5-T was added. The extract was decanted into a 250-mL flask through a funnel plugged with cotton, and the pad extraction was repeated 2 more times with 25 mL each of MeOH. After the last extract had been filtered, the funnel was rinsed with three 5.0-mL rinses of MeOH, and the combined extracts and rinses were reduced to about 10 mL by gently boiling on a hot plate. The extract was quantitatively transferred to a 50-mL flask with 15 mL of MeOH and analyzed by the procedure described under Methylation and GLC Analysis.

Analysis of Samples from Hand Rinses. Each hand of the worker was placed in a plastic bag and 150 mL of 95% EtOH was added. The bags were tightly secured around the wrist and shaken vigorously for 30 s. The bags containing the extract were placed in widemouth $^1/_2$-pt jars and transported to the laboratory. In the laboratory, the bags were opened and the extracts decanted into bottles and stored in a refrigerator until analysis.

When analysis was scheduled, the bottles were removed from the refrigerator and allowed to warm to room temperature. If the concentration of 2,4-D in a sample was expected to be high (>0.1 μg of 2,4-D/mL of hand rinse), a 10-mL portion was pipetted into a flask containing 30 mL of water, 0.5 μg of 2,4,5-T, and 2.0 mL of aqueous 37% KOH (w/v). If the concentration of 2,4-D was expected to be near the limits of detection (0.03 μg of 2,4-D/mL of hand rinse), a 100-mL portion of the sample was transferred to a flask containing 30 mL of water, 0.5 μg of 2,4,5-T, and 2.0 mL of 37% KOH. In either case, the solution in the flask was reduced to about 20 mL by boiling on a hot plate, cooled, and then acidified with 2.0 mL of concentrated H_3PO_4. For cleanup, the solution was drawn at a flow rate of about 5 mL/min through a Sep-PAK cartridge with the aid of vacuum from a water aspirator. The flask was rinsed with three 5-mL rinses of acidified water, and each of the rinses were drawn through the Sep-PAK. The Sep-PAK was removed from vacuum and attached to a 10-mL hypodermic syringe and rinsed with 10 mL of hexane. The rinse was discarded. Residues of 2,4-D and the 2,4,5-T identifier were eluted from the Sep-PAK into a 50-mL flask with 10 mL of MeOH, and the analysis was continued by the procedure described under Methylation and GLC Analysis. The Sep-PAKs were regenerated and saved for later use.

Analysis of Urine. Urine samples obtained from workers exposed to 2,4-D formulations were frozen. Prior to analysis, samples were warmed to room temperature, and any sediment was dispersed by shaking. A portion of the 2,4,5-T (0.5 μg) was added to a 250-mL flask, and the solvent was evaporated with a stream of air. A 15-mL portion of urine was transferred to the flask, and 15 mL of water, 2.0 mL of 37% KOH, and glass beads were added. The sample was boiled on a hot plate for 15 min, cooled, and acidified with 2.0 mL of concentrated H_3PO_4. The solution was then drawn through a Sep-PAK as previously described for hand-rinse samples. The sample flask was rinsed with three 5-mL rinses of acidified water, and each of the rinses were drawn through the Sep-PAK. The Sep-PAK was detached from the vacuum and treated as described for hand-rinse samples. The Sep-PAKs used for these analysis were regenerated for reuse.

Analysis of Perspiration Samples. Perspiration from workers who had been exposed to 2,4-D was collected in absorbent cotton T-shirts. The workers wore the shirts as undergarments and changed them every 24 h. Disposable gloves were worn when changing the shirts to prevent hand contact with the shirt. Before use, the shirts were laundered with soap and water, extracted with MeOH in a Soxlet for 18 h, and then dried in a household clothes dryer.

Residues of 2,4-D were extracted by placing each shirt in a 2-L jar containing the dried residue of 1.0 μg of the 2,4,5-T identifier standard and 1 L of water. The jar was sealed and tumbled for 1 h. A portion of the extract in excess of 250 mL was filtered under vacuum through a filter paper. A 250-mL portion of the filtrate was transferred to a flask, acidified with 10 mL of H_3PO_4, and drawn through a Sep-PAK as described for the hand-rinse samples. The sample flask was rinsed with three 10-mL portions of acidified water and each rinse was drawn through the Sep-PAK. The Sep-PAK was detached from the vacuum and the 2,4-D and 2,4,5-T were eluted by the procedure described for hand-rinse samples. The Sep-PAKs used in the cleanup of these samples could not be regenerated and therefore were discarded.

Methylation and GLC Analysis. The residues of 2,4-D in MeOH were esterified or transesterfied by adding 1 mL of the 14% BF_3 reagent and boiling on a hot plate. When the MeOH was essentially boiled away and the remaining liquid become viscous and white vapors formed in the flask, the sample was removed from the hot plate, 1 mL of water was added, and the solution was placed in a refrigerator to cool. Then 10 mL of hexane was added, the flask was stoppered, and the solution was shaken vigorously for 15 s. When the phases had separated, a 6.0-mL aliquot of the hexane solution was transferred to a vial and evaporated to dryness with a gentle stream of air. The residue was dissolved in an appropriate volume of hexane and stored in a refrigerator until analysis by GLC.

RESULTS AND DISCUSSION

Since Pursley and Schall (1965) demonstrated that various esters of 2,4-D could be directly transesterified to the methyl ester with a MeOH solution of BCl_3, and Horner et al. (1974) demonstrated that a MeOH solution of BF_3 could be used to methyl esterify 2,4-D, consideration was given to the possibility of using BF_3 in MeOH to directly convert 2,4-D, the dimethylamine salt, and various esters to the methyl ester. Therefore, the methylation procedure described in this paper was applied to pure standards of the dimethylamine salt, isooctyl ester, butoxyethanol ester, and 2,4-D acid. The resultant methyl esters were compared with a commercially available analytical standard of 2,4-D methyl ester (99.5% purity). The comparison showed that the 2,4-D, its esters, and its dimethylamine salt were quantitatively converted to the methyl ester by this procedure. Horner et al. (1974) refluxed 2,4-D in MeOH with BF_3 for 10 min and removed the MeOH under vacuum. In our work, the 2,4-D and its esters and dimethylamine salt were converted to the methyl ester by boiling them with the BF_3–MeOH on a hot plate. This procedure not only removed the MeOH but also facilitated the direct transfer of the 2,4-D methyl ester to hexane, saving considerable time. This procedure allowed residues of 2,4-D or its esters or dimethylamine salt in MeOH extracts of dermal exposure pads to be converted to the methyl ester and the MeOH evaporated in one step. Care must be taken to avoid boiling the BF_3–MeOH solution beyond the point described under Methylation and GLC Analysis, as this will result in low recoveries. One milliliter of the 14% BF_3 was adequate

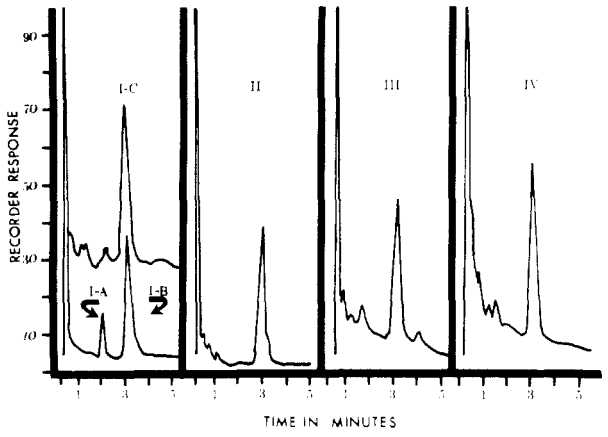

Figure 1. GLC chromatograms of methylated standards of 0.2 ng of 2,4-D (IA) and 0.5 ng of 2,4,5-T (IB) and of control samples of an extract of an exposure pad equivalent to 34 cm^2 (IC), a hand rinse equivalent to 100 mL of extract (II), a urine equivalent to 15 mL (III), and a T-shirt extract equivalent to 250 mL (IV). All control sample chromatograms are the result of the analytical procedures described in this paper and all were fortified with 2,4,5-T prior to analysis.

for the methyl esterification of up to 1.0 mg of 2,4-D.

Analysis of control samples of dermal exposure pads, hand rinses, urine, and T-shirts obtained from workers before exposure to 2,4-D contained no detectable amount of 2,4-D methyl ester or any contaminant with the same retention time (Figure 1). The lower limit of detection of 2,4-D residues was 0.02 $\mu g/cm^2$ of dermal exposure pads, 0.07 μg/mL of urine, 4.5 μg/hand rinse, and 1.0 μg/T-shirt. The 2,4,5-T peak shown in Figure 1 was the result of adding this compound to all samples as an identifier, to determine if any qualitative errors were made in analysis of these samples, and was not used to calculate the efficiency of the method. Analytical efficiency was determined by adding known amounts of the pure 2,4-D, its esters, or its dimethylamine salt to samples prior to analysis and then determining the percent recovery. These results are shown in Table I.

In human blood, the 2,4-D ester is converted to the acid or an acid–protein conjugate and eliminated from the body through urinary excretion (Leng, 1977). To maximize the recovery of 2,4-D from urine, Glas (1978) hydrolyzed the acid–protein conjugate by heating the urine for 90 min with KOH. Thus, a study was conducted to determine the most efficient hydrolysis time. Aliquots of urine (15 mL) or T-shirt extracts (50 mL) were taken from single homogeneous samples so that errors due to sample variation would not be a factor. Comparison of results obtained from this experiment (Table II) shows that there was no significant difference in the yield of 2,4-D residues found in urine or perspiration samples not treated with KOH and those boiled with KOH over a range of 0–90 min. Boiling the urine for 15 min with KOH produced GC traces with fewer extraneous peaks. Therefore, urine samples were hydrolyzed routinely.

The cleanup procedure using reverse-phase liquid chromatographic support was satisfactory for residues of 2,4-D or its dimethylamine salt, but esters of 2,4-D could not be recovered with this procedure. Determination of residues of 2,4-D ester were needed in the analysis of hand-rinse samples. Therefore, before cleanup, all hand rinses were boiled with KOH to hydrolyze ester residues to 2,4-D. Boiling also removed the EtOH which would otherwise elute 2,4-D from the Sep-PAK.

A single Sep-PAK could be easily regenerated and reused up to 5 times each for urine or hand-rinse analyses (Table III). However, Sep-PAKs could not be regenerated after analysis of perspiration samples. Because of the possibility that the Sep-PAKs regenerated after use in the analysis of high-residue hand-rinse samples may still contain traces of 2,4-D, these Sep-PAKs were used to analyze control samples of urine and T-shirt extracts. The GLC chromatograms showed no traces of 2,4-D residues and were typical of the ones shown in Figure 1 for urine and T-shirt extract control samples.

Table I. Recovery of 2,4-D, Its Esters, or Its Dimethylamine Salt from Exposure Pads, Hand Rinses, T-shirts, and Urine

sample	sample size	amount added per sample, μg	no. of analyses	% recovery found ± SD
		2,4-D		
exposure pads	42.3 cm^2	5.0	3	88.3 ± 11.2
		10.0	2	82.0
hand rinses	150 mL	10.0	2	88.5
		20.0	2	77.5
T-shirt	250 mL	2.0	1	105.0
		5.0	12	98.3 ± 17.3
		10.0	15	88.6 ± 19.4
		20.0	10	74.3 ± 34.1
urine	15 mL	1.0	1	80.0
		5.0	19	84.1 ± 22.3
		10.0	17	80.2 ± 22.9
		2,4-D Isooctyl Ester		
exposure pads	42.3 cm^2	1.0	3	71.0 ± 1.0
		10.0	3	80.0 ± 4.4
		200.0	2	84.0
hand rinses	150 mL	10.0	7	77.0 ± 1.4
		200.0	4	81.0 ± 8.2
		2,4-D Butoxyethanol Ester		
exposure pads	42.3 cm^2	1.0	4	68.0 ± 5.0
		5.0	8	88.8 ± 24.3
		10.0	9	85.9 ± 18.8
		20.0	3	82.0 ± 3.6
		100.0	4	82.8 ± 10.2
hand rinses	150 mL	5.0	2	68.0
		30.0	4	78.0 ± 3.6
		100.0	2	72.0
		200.0	2	74.5
		500.0	6	74.8 ± 5.7
		2,4-D Dimethylamine Salt		
exposure pads	42.3 cm^2	1.0	10	83.4 ± 19.4
		5.0	20	92.1 ± 21.1
		10.0	27	89.4 ± 24.8
		100.0	3	102.0 ± 15.4
		200.0	2	86.0
hand rinses	150 mL	5.0	14	80.2 ± 14.6
		10.0	35	87.7 ± 12.1
		20.0	8	79.3 ± 14.3
		100.0	6	110.8 ± 17.2
		200.0	23	87.4 ± 17.2
		400.0	6	91.7 ± 13.2

Table II. Relation between Duration of KOH Hydrolysis and the Yield of 2,4-D Residues from Urine and Perspiration

duration of hydrolysis, min	yield of 2,4-D residues: urine, ppm, sample 1	urine, ppm, sample 2	perspiration, μg/shift
control[a]	2.60	0.13	16.7
0	2.78	0.15	17.5
15	3.15	0.14	15.5
30	3.20	0.13	17.0
60	2.58	0.14	17.0
90	1.14	0.11	16.5

[a] The control samples were not hydrolyzed.

Table III. Recovery of Residues of 2,4-D from Urine and Hand-Rinse Samples after Regeneration and Reuse of Sep-PAK Cartridge

no. of times Sep-PAK used	% recovery	
	urine (0.5 μg/mL)[a]	hand rinses (2.0 μg/mL)[b]
1	88 ± 8	94 ± 2
2	93 ± 4	86 ± 8
3	94 ± 9	80 ± 5
4	90 ± 9	95 ± 4
5	90 ± 13	100 ± 0

[a] These results are the average of four analyses ± standard deviation. [b] These results are the average of three analyses ± standard deviation.

This work demonstrates a procedure for the efficient determination of residues of 2,4-D and its esters and dimethylamine salt in dermal exposure pads, hand-rinse samples, urine, and perspiration samples based on direct methylation of these compounds with BF_3 and the rapid cleanup of samples with a commercially available cartridge of reverse-phase liquid chromatographic support (Sep-PAK). At present, this procedure is being successfully applied to the analysis of exposure samples collected from monitoring studies of agricultural workers involved in the application of these compounds. The results of these studies will be published later.

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Wild Oat Herbicide Studies. 3. Physiological and Biochemical Bases for Interaction of Barban and Growth Regulator Herbicides in Wild Oat

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The instability of barban (4-chloro-2-butynyl *m*-chlorocarbanilate) in tank mixtures with growth regulator herbicides was not a factor contributing to the antagonism between barban and growth regulator herbicides. The antagonism occurred within the plant. Of five growth regulator herbicides, the antagonism was greatest in the mixture of barban/2,4-D[(2,4-dichlorophenoxy)acetic acid] isopropyl ester and 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] triethylamine/barban. Penetration and translocation of [^{14}C]barban in the leaves and to the meristematic tissue of the growing point were inhibited by 2,4-D ester. The meristematic tissue was stimulated to produce [^{3}H]DNA from a precursor, [^{3}H]thymidine, by 2,4-D ester, but inhibited by barban and the barban/2,4-D mixture. It may be concluded that the reduction of barban activity in wild oat (*Avena fatua* L.) by the growth regulator herbicides appeared to be associated with two factors, (1) physiologically reduced penetration and translocation of barban in the leaves to the meristematic tissue of the growing point and (2) biochemically stimulated DNA synthesis by 2,4-D to counteract the inhibitory effect of barban in meristematic tissue.

An ideal herbicide spray effectively controls grass and broadleaf weeds simultaneously. This may be achieved by using a herbicide mixture because most herbicides developed as grass killers control wild oat only. Considerable interest has been shown in package mixtures in recent years. However, herbicides such as barban in mixture with a growth regulator herbicide often reduces wild oat control (Holroyd, 1960; Pfeiffer et al., 1960). The interaction which reduces weed control in herbicide mixtures is defined as antagonism (Colby, 1967).

The metabolism of barban (Shimabukuro et al., 1976; Still and Mansager, 1972) and selectivity of barban between oat or wild oat and cereal crops have been studied (Jacobsohn and Andersen, 1972; Kobayashi and Ishizuka, 1974; Shimabukuro et al., 1976). Barban inhibited synthesis of proteins and nucleic acids (Chow, 1982; Kobayashi

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