Gas Chromatographic Determination of Erbon and Two Metabolites in Biological Materials

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Gas chromatographic methods for determining erbon [2(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate] and two metabolites [2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol] in blood, urine, feces, and tissues are presented. After extraction, the herbicide and metabolites are concentrated in a small volume of hexane. The metabolites are reacted to produce trimethylsilyl derivatives prior to analysis. Average recoveries of about 77% were obtained from tissues fortified with known amounts of erbon. Recoveries from samples fortified with 2,4,5-trichlorophenol averaged 41% for blood, 77% for urine, and about 90% for feces and tissue. Average recoveries from samples fortified with 2(2,4,5-trichlorophenoxy) ethanol were 72% for blood, 100% for urine, and 95% for feces and tissues. Minimum detectable quantities of the parent compound and metabolites are given for blood, urine, feces, and muscle.

Erbon [2(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate] is an herbicide that is used to control grass and weeds in noncrop areas. Erbon, when administered orally to sheep, is metabolized rapidly to 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol (Wright *et al.* 1969). *In vitro* the herbicide is metabolized to 2(2,4,5-trichlorophenoxy) ethanol in urine, feces, and blood (Wright *et al.* 1969). The chemical structures for these compounds are shown in Figure 1.

Since the possibility of livestock consuming forage that has been sprayed with erbon exists, a residue study of this compound and some of its metabolites in biological materials is warranted.

This paper presents procedures for the extraction of erbon and two of its metabolites from biological materials with quantitation by gas chromatography.

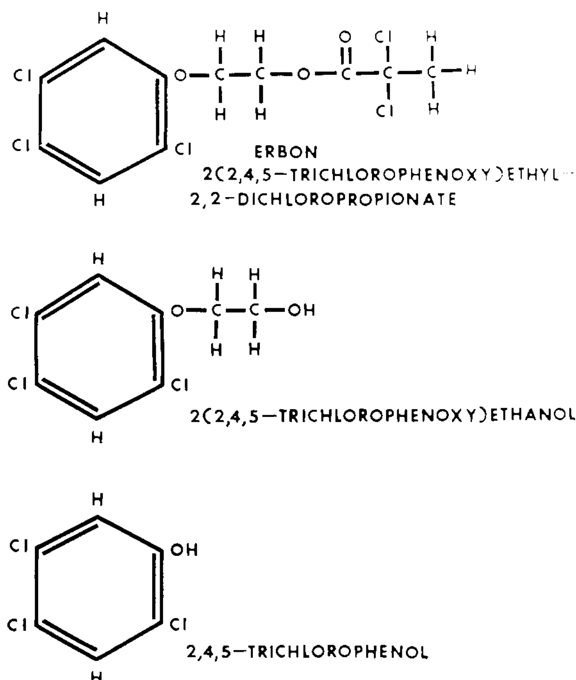

Figure 1. Chemical structure of Erbon and metabolites

MATERIALS AND METHODS

Reagents. Erbon. (Baron) 2(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate technical standard (Dow Chemical Co., Midland, Mich.)

Metabolites. 2(2,4,5-trichlorophenoxy) ethanol, 6.34% OH, and 2,4,5-trichlorophenol, 98.0% Analytical standard (Dow Chemical Co., Midland, Mich.).

Silyl-8. GLC Column Conditioner (Pierce Chemical Co., Rockford, Ill.)

Tri-Sil Concentrate. Reagent-Catalyst Formulation (Pierce Chemical Co., Rockford, Ill.)

Apparatus. Chromatograph. Micro-Tek 220 equipped with Nickel-63 electron capture ionization detector with pulse power supply. (Micro-Tek, Austin, Texas).

Tissue Grinders. Thomas (Matheson Scientific, Houston, Texas).

Standard Preparation. A series of erbon standards (ranging in concentration from 0.2 to 1.0 ng. per μl.), 2,4,5-trichlorophenol standards (0.02 to 0.2 ng. per μl.), and 2(2,4,5-trichlorophenoxy) ethanol standards (0.2 to 1.0 ng. per μl.) were prepared in redistilled hexane. A standard curve for erbon was determined by injecting 2-μl. portions into the gas chromatograph. The electron capture detector used in this study was not adequately sensitive to the metabolites. Therefore, to improve the sensitivity of the gas chromatographic detector to the metabolites, 1.0-ml. portions of each were reacted with 0.25 ml. of Tri-Sil Concentrate. The sample was allowed to stand 5 minutes for complete reaction. The detector sensitivity to the derivatives was increased 10-fold for 2,4,5-trichlorophenol and 100-fold for 2(2,4,5-trichlorophenoxy) ethanol. (Erbon is unaffected by Tri-Sil Concentrate.) Standard curves for both metabolites were determined by injecting 2-μl. portions of the trimethylsilyl derivatives into the gas chromatograph for analysis. Standards of these three compounds, erbon and metabolites, in petroleum ether behave similarly.

Collection of Samples. All samples were obtained from clinically normal sheep in good condition. Blood samples were obtained by direct puncture of the jugular vein and were heparinized immediately. Urine samples were obtained by catheterization. Fecal samples were collected over a 6-hour

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Table I. Average Recovery of Erbon from Biological Materials[a]

Material	Erbon Added P.P.M.	P.P.M. Erbon Recovered as Erbon	Metabolite 1[b]	Metabolite 2[c]	Total Recovered P.P.M.	Per Cent Recovered
Fluids						
Blood	0.05	...	...	0.02	0.02	46.7
	0.50	...	...	0.45	0.45	90.5
Excreta						
Urine	0.05	...	...	0.05	0.05	100.0
	0.50	...	...	0.56	0.56	112.0
Feces	1.00	...	0.07	0.57	0.64	64.0
Tissues						
Brain	4.00	...	0.06	3.02	3.08	77.0
Adipose, Omental	4.00	2.30	...	0.58	2.88	72.0
Adipose, Subcut.	4.00	2.56	...	0.36	2.92	73.0
Heart	4.00	2.52	...	0.55	3.07	76.8
Intestine, Small	4.00	3.02	...	1.31	4.33	108.3
Kidney	4.00	0.80	...	2.47	3.27	81.8
Lung	4.00	0.66	...	2.26	2.92	73.0
Muscle	0.50	0.13	...	0.24	0.37	72.8
	1.00	0.62	...	0.20	0.82	81.9
	4.00	2.58	...	0.64	3.22	80.5
Spleen	4.00	0.68	...	1.18	1.86	46.5
Thyroid	4.00	2.58	...	0.70	3.28	82.0

[a] All samples were run in duplicate.
[b] 2,4,5-trichlorophenol.
[c] 2(2,4,5-trichlorophenoxy) ethanol.

period and combined. Tissue samples were obtained upon sacrifice of the animal, ground with an electric meat grinder, and frozen until use.

Extraction. BLOOD. A modified procedure of Dale *et al.* (1966) was used for the extraction of the herbicide and two metabolites from blood. For the extraction of erbon and 2(2,4,5-trichlorophenoxy) ethanol, 2 ml. of blood was pipetted into a 15-ml. centrifuge tube. Five milliliters of hexane was added and the sample shaken manually for 3 minutes. The sample was centrifuged at 2000 r.p.m. to break the emulsion, if necessary. The organic layer was removed and dried with a small amount of Na_2SO_4. Erbon concentrations were determined by gas chromatography. The solutions were concentrated, if necessary, to obtain adequate response. A 4-ml. portion of the extract was evaporated to dryness, the residue taken up in 100 μl. of hexane, and reacted with 25 μl. of Tri-Sil Concentrate to produce a trimethylsilyl derivative of 2(2,4,5-trichlorophenoxy) ethanol. After 5 minutes, 2 μl. of the reaction mixture was injected into the gas chromatograph for quantitation. (Reacted samples are stable for 5 days or longer.)

For extraction of 2,4,5-trichlorophenol, 2 ml. of blood was pipetted into a 15-ml. centrifuge tube; 5 ml. of distilled H_2O and 1 ml. of 1*N* HCl was added. The solution was mixed well and allowed to stand at room temperature for 1 hour. This mixture was extracted with 5 ml. of hexane and analyzed as for 2(2,4,5-trichlorophenoxy) ethanol.

URINE. A modified procedure of Dale *et al.* (1966) was used for the extraction of the parent compound and metabolites from urine. The total urine sample was incubated at 37° C. for 7 days. (Ambient temperature and longer periods of time may be used.) Then 2 ml. of the incubated urine was pipetted into a centrifuge tube and extracted with hexane for 2(2,4,5-trichlorophenoxy) ethanol as in blood for quantitation. To extract 2,4,5-trichlorophenol, the aliquot was made acid with HCl and then extracted as usual. For *in vitro* studies, erbon added to urine metabolizes rapidly to 2(2,4,5-trichlorophenoxy) ethanol and the metabolite is extracted without this incubation step. For *in vivo* studies, erbon appears in the urine as both 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol after the incubation step. No erbon was found in the urine at any time.

FECES. Total feces was weighed and placed in blender. For each gram of feces, 3 ml. of distilled H_2O was added. The water-feces mixture was blended at high speed for 1 to 2 minutes. A 4-gram portion of the mixture was weighed immediately and transferred to a 250-ml. separatory funnel with 100 ml. of distilled H_2O. The mixture was extracted three times with 50-ml. portions of hexane for 2(2,4,5-trichlorophenoxy) ethanol and the parent compound. To extract the 2,4,5-trichlorophenol from the feces, the mixture was made acid with HCl prior to the extractions with hexane. The combined emulsions were transferred to centrifuge bottles and centrifuged for approximately 10 minutes at 3000 r.p.m. and 5° C. The centrifuged sample was filtered through doubled cheesecloth into a clean separatory funnel. The aqueous layer was discarded. The hexane was concentrated to 1 ml. and the presence of erbon checked by gas chromatography. (Both metabolites were lost partially during evaporation so extreme care must be taken to keep this at a minimum.) The samples were reacted with Tri-Sil Concentrate and analyzed for trimethylsilyl derivatives of the metabolites as described earlier.

TISSUE SAMPLES. A modified procedure of Radomski *et al.* (1967) was used for the extraction of the herbicide and metabolites from tissues. Tissue samples of 250 mg. were weighed into tissue grinders and ground well with 3 ml. of petroleum ether. The tissue to be extracted for 2,4,5-trichlorophenol was made acid with HCl prior to extraction with petroleum ether. The extraction solvent was transferred to a 15-ml. centrifuge tube. The tissue was re-extracted with a small volume of petroleum ether. The extraction solvents were combined and brought to a 5-ml. volume and mixed well. The hexane extract was dried with sodium sulfate and then centrifuged if cloudy. The presence of erbon was checked. The extract was concentrated, reacted with Tri-Sil

Table II. Average Recovery of 2,4,5-Trichlorophenol from Biological Materials[a]

Material	Amount Added P.P.M.	Amount Recovered P.P.M.	Per cent Recovered
	Fluids		
Blood	0.025	0.008	32.8
	0.05	0.025	49.3
	Excreta		
Urine	0.0125	0.009	70.5
	0.125	0.104	83.2
Feces	0.05	0.03	66.0
	0.10	0.04	40.4
	0.20	0.18	90.0
	Tissues		
Adrenal	4.00	3.37	84.2
Brain	4.00	4.12	103.6
Adipose, Omental	4.00	4.49	112.3
Adipose, Renal	4.00	4.38	109.6
Adipose, Subcutaneous	4.00	4.07	101.8
Heart	4.00	3.40	85.0
Intestine, Small	4.00	3.28	82.1
Kidney	4.00	3.23	80.8
Liver	4.00	2.50	62.5
Lung	4.00	3.36	83.9
Muscle	0.10	0.047	47.0
	0.20	0.13	63.0
	4.00	3.28	82.1
Spleen	4.00	3.40	85.0
Thyroid	4.00	3.50	87.5

[a] All samples run in duplicate.

Table III. Average Recovery of 2(2,4,5-Trichlorophenoxy) Ethanol from Biological Materials[a]

Material	Amount Added P.P.M.	Amount Recovered P.P.M.	Per Cent Recovered
	Fluids		
Blood	0.025	0.018	72.6
	Excreta		
Urine	0.05	0.06	119.1
	0.50	0.53	106.5
Feces	0.05	0.03	60.0
	0.10	0.06	58.0
	1.00	0.996	99.6
	Tissues		
Adrenal	20.00	17.34	86.7
Brain	4.00	3.01	75.2
Adipose, Omental	4.00	4.48	112.0
Adipose, Renal	4.00	3.83	95.8
Adipose, Subcutaneous	4.00	3.84	96.0
Heart	4.00	3.99	99.8
Intestine, Small	4.00	3.36	84.0
Kidney	4.00	4.19	104.8
Liver	4.00	3.58	89.4
Lung	4.00	3.84	96.0
Muscle	0.125	0.075	60.0
	0.25	0.125	50.0
	4.00	3.58	89.4
Spleen	4.00	3.84	96.0
Thyroid	4.00	4.38	109.4

[a] All samples were run in duplicate.

Concentrate, and then checked for trimethylsilyl derivatives of the metabolites by gas chromatography.

Gas Chromatographic Analysis. The following conditions were used.

Column. Stainless Steel, 6-foot × 1/4-inch O.D.

Packing. Dow 11 silicone, 5% on 60- to 80-mesh chromosorb W.

Carrier Gas. Nitrogen at 100 ml. per minute.

Temperatures. Column 215° C. for erbon and 180° C. for the trimethylsilyl derivatives of 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol; injection port 275° C.; detector 270° C.

The column was conditioned at 275° C. for at least 24 hours prior to use in analyses. Two microliters of each sample (or an appropriate dilution thereof if too concentrated) was injected for analysis. Response to the compounds in the extracts and the standards was measured in terms of peak height.

The retention time for the parent compound, erbon, was 3.2 minutes (215° C.) using the above mentioned parameters. The retention times for the trimethylsilyl derivatives of 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol were 1.0 and 3.2 minutes (180° C.), respectively.

DISCUSSION

When erbon is added to blood, urine, or feces and allowed to stand at room temperature for at least one hour, total metabolism takes place rapidly in which 2(2,4,5-trichlorophenoxy) ethanol is produced. When administered orally, erbon appears in the blood, urine, and feces as 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol. Metabolism also occurs when erbon is added to tissue, but is generally not as rapid or complete as other reactions (Table I). Recovery of erbon from spiked samples ranged from 46.5% for spleen to 100% for urine. The metabolite, 2(2,4,5-trichlorophenoxy) ethanol, accounts for the major portion of erbon recovered from blood, urine, and feces.

The metabolite, 2,4,5-trichlorophenol, is recovered from spiked biological samples in good quantity with the exception of blood (Table II). Recovery from blood averaged approximately 41.0% at low levels. Recovery from urine, feces, and tissues (4 p.p.m.) averaged 76.9, 90.5, and 89.3%, respectively.

Good recovery is obtained for 2(2,4,5-trichlorophenoxy) ethanol when extracting spiked biological samples (Table III). Average recovery from blood, urine, feces, and tissues (4 p.p.m.) was 72.3, 112.8, 99.6, and 95.0%, respectively.

The described method will detect erbon in concentrations as low as 0.05 p.p.m. in blood and urine, and 0.5 p.p.m. in muscle and feces. Concentrations of 2,4,5-trichlorophenol as low as 0.03 p.p.m., 0.05 p.p.m., 0.01 p.p.m., and 0.1 p.p.m. were detected in blood, feces, urine, and muscle, respectively. The method detected concentrations of 2(2,4,5-trichlorophenoxy) ethanol as low as 0.03 p.p.m. in blood, 0.05 p.p.m. in urine and feces, and 0.13 p.p.m. in muscle.

After repeated injections of tissue extracts, the erbon peak sometimes appears as a double peak on the chromatogram. In our work, this was remedied by the injection of 10 μl. of Silyl-8 to recondition the column.

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