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Short Communication

Determination of the herbicide diclofop in human urine

Allan J. Cessna* and Raj Grover

Agriculture Canada, Research Station, Regina, SK S4P 3A2 (Canada)

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ABSTRACT

A simple and sensitive method for the gas chromatographic determination of diclofop residues in human urine is described. Recoveries of diclofop, as its methyl ester, from fortified urine were greater than 85% at 100, 50, 10 and 1 $\mu g\ kg^{-1}$, and were similar with and without the inclusion of a hydrolytic step in the analytical method. However, a hydrolytic step was necessary for analysis of 24-h urine samples collected from a male applicator following a single exposure to diclofop-methyl during application to wheat using a tractor-pulled sprayer. Diclofop residues determined with hydrolysis were approximately double those without hydrolysis, suggesting that a significant portion of diclofop was excreted in the conjugated form.

INTRODUCTION

Applicators are exposed to pesticides both dermally and by inhalation [1,2] resulting in absorption of these chemicals into the body both through the skin [3] and the respiratory tract [4]. When excreted in the urine, the cumulative urinary excretion of pesticides provides an indirect estimate of the amount of pesticide which entered the body.

Conjugation of acidic herbicides, such as (2,4-dichlorophenoxy)acetic acid (2,4-D) [5], (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) [6] and (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid (diclofop) [7], is now a well-established metabolic pathway in plants and has also been recognized in animals. For example, conjugates of 2,4-D and 2,4,5-T have been found in the urine of pigs [8] and humans [9], and rats [10], respectively. Thus, in order to accurately determine cumulative urinary excretion of acidic herbicides, such conjugates must be cleaved by the analytical method prior to quantitation. Previous studies involving the urinary excretion of acidic herbicides have included a hydrolytic step to cleave possible herbicide conjugates [11–13].

Several acidic herbicides, such as 2,4-D [11,14,15], (4-chloro-2-methylphenoxy)acetic acid (MCPA) [15], 2,4,5-T [16], (±)-2-(2,4-dichlorophenoxy)propanoic acid (dichlorprop) [15], (±)-2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop) [15] and 3,6-dichloro-2-methoxybenzoic acid (dicamba) [14], are known to be excreted in the urine of exposed applicators. Diclofop (Fig. 1) is an acidic herbicide which is extensively applied as its methyl ester (diclofop-methyl) for the post-emergent control of wild oats (*Avena fatua* L.) and other grassy weeds in a variety of crops. However, there are no published data available regarding human urinary excretion of diclofop following exposure. The objectives of the

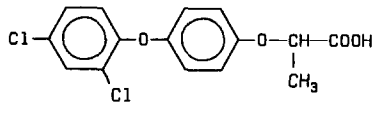

Fig. 1. Structural formula for diclofop.

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present study were (i) to develop, for use in a subsequent applicator exposure study, a sensitive analytical method for the determination of diclofop residues in human urine which included a hydrolytic step to cleave possible conjugates and (ii) to determine whether diclofop conjugates were present in urine collected from an exposed applicator by analysis with and without hydrolysis.

EXPERIMENTAL

Materials

All solvents were distilled-in-glass grade (Caledon Laboratories). The Florisil (Fisher Scientific) was heated at 600°C for 48 h and then deactivated by the addition of 5% (w/w) water. The sodium sulphate was heated at 600°C for 24 h. Deionized water was obtained using the Nanopure II cartridge system (Barnstead). The boron trifluoride–methanol reagent was 14% (w/w) BF_3 (BDH). Analytical standards of diclofop and diclofop-methyl were obtained from Hoechst Canada (Regina, Canada).

Urine collection

Two volunteers, who were not involved in any spray operations, provided urine samples for method development. Later, they also participated as controls by alternately providing 24-h urine samples every second day throughout the 12-days experimental period during the spring spraying season, and also for a 10-days period after the spraying season ended to ascertain background levels.

In order to test the effectiveness of the developed method with and without hydrolysis, composite 24-h urine samples were collected from an exposed applicator beginning 1 day before spraying and then continuously until 10 days after spraying. The samples were collected such that the first void of the day was included as part of the previous day's sample.

All urine voids for each 24-h period were collected in disposable polyethylene-lined, 2.5-l urine specimen storage containers (Fisher Scientific, Cat. No. 14-375-119). The containers were collected and dated each day, and then stored at −10°C until extraction.

Field procedures

The farmer applicator (body weight 109 kg) who provided "exposed" urine samples followed his normal mixing and spraying procedures and did not wear protective gloves or a respirator. Diclofop-methyl [624 g acid equivalent (a.e.) ha^{-1}], tank mixed with bromoxynil (3,5-dibromo-4-hydroxybenzonitrile, 247 g phenol equivalent ha^{-1}), was applied in 110 l water ha^{-1} to 36.4 ha of wheat at the 3- to 4-leaf stage using a tractor-pulled sprayer. The sprayer, equipped with a 910-l tank, a 13-m boom and 28 Tee-jet 6502 nozzles, was operated at a pressure of 275 kPa using a boom height of 51 cm above the crop canopy and a ground speed of 9 km h^{-1}. The tractor used to pull the sprayer was equipped with a closed cab which provided filtered air (dust filter only). The spraying operation lasted 550 min, during which the sprayer tank was filled four times.

Non-hydrolytic extraction

Urine (100 ml), contained in a 250-ml separatory funnel, was acidified to pH 1–2 by the addition of 3 *M* H_2SO_4 and extracted twice with 100-ml portions of methylene chloride. Each methylene chloride extract was passed through 25 ml of anhydrous sodium sulphate (contained in a 9-cm diameter long-stemmed funnel on top of a glass wool plug) into a 250-ml round-bottom flask, followed finally by a 25-ml methylene chloride wash of the sodium sulphate. The combined extracts were taken just to dryness using a rotary evaporator and the extract residue transferred with three 1.5-ml portions of methanol to a 150 mm × 18 mm I.D. test tube.

Boron trifluoride–methanol methylation

Boron trifluoride reagent (4.5 ml) was added to the test tube and the tightly stoppered test tube was heated in a dry block heater at 70°C for 1.5 h. The tube was then cooled by emmersing in an ice–water bath. Saturated NaCl solution (10 ml) and hexane (10 ml) were added and the stoppered tube shaken vigorously for 1 min. The organic layer was transferred by using a disposable Pasteur pipette to a test tube containing 2 g of anhydrous sodium sulphate. The extraction of the aqueous solution was repeated with a further 10 ml of hexane. The combined hexane extracts were decanted from the drying agent into a 40-ml centrifuge tube together with a 5-ml hexane rinse of the sodium sulphate. The hexane solution was evaporated to approximately 1 ml

using a stream of nitrogen gas and the concentrated extract subjected to Florisil column cleanup.

Florisil column cleanup

Florisil (4 ml) was added to a 200 mm × 10 mm I.D. column containing 10 ml of hexane, and topped with 1 cm of anhydrous sodium sulphate. The hexane was drained to the surface of the sodium sulphate and the methylated extract was transferred to the column, including two 1.5-ml hexane rinses of the centrifuge tube. The column was then eluted with 60 ml of 0.5% acetone in hexane solution, the last 48 ml of which were concentrated to approximately 0.5 ml using a stream of nitrogen gas and then taken to volume (1 ml) with hexane.

Hydrolytic extraction

Sodium hydroxide solution (10 ml, 10 *M*) was added to 100 ml of urine contained in a 250-ml flat-bottom flask and the contents gently refluxed (6-unit Vari-heat extraction rack, GCA Corp.) for 4 h. After cooling, the condenser was rinsed with water and then 3 *M* H_2SO_4 (25 ml) was used to acidify the solution (pH 1–2). The acidified urine sample was then extracted with methylene chloride and subsequent sample workup carried out as described above.

Gas chromatographic system

An Hewlett-Packard Model 5890A gas chromatograph, equipped with a ^{63}Ni electron-capture detector and an on-column injector, was used with the Model 7673A autosampler set to inject 2 μl, and the Model 5895A data station. A Hewlett-Packard 30 m × 0.53 mm I.D. HP-1 (film thickness, 0.88 μm) fused-silica column was used with the following temperature program: 70°C for 1 min, then 10°C min^{-1} until 270°C, and hold for 1 min. Using a carrier gas (helium) flow-rate of 5 ml min^{-1} and detector make-up gas (nitrogen) flow-rate of 70 ml min^{-1}, diclofop methyl ester had a retention time of 12.82 min.

Fortification

Urine (250 ml), collected for method development and contained in the polyethylene-lined urine speci-

TABLE I

RECOVERIES OF DICLOFOP FROM FORTIFIED URINE SAMPLES COLLECTED FROM NON-EXPOSED VOLUNTEERS USING HYDROLYTIC AND NON-HYDROLYTIC PROCEDURES

Fortification level ($\mu g\ l^{-1}$)	Sample		Diclofop recovery (%)	
	Volunteer	Date	With hydrolysis	Without hydrolysis
100	2	June 16	88.3	80.4
	2	July 10	117.8	–
	2	July 14	82.4	–
	1	July 20	78.9	77.4
50	2	June 12	77.4	86.8
	1	July 12	83.3	–
	1	July 16	74.2	78.4
	1	July 20	80.6	–
10	1	June 6	89.1	–
	2	June 16	90.2	–
	2	July 10	–	95.3
	1	July 12	81.1	–
	1	July 16	85.1	97.8
1	1	July 10	90.3	–
	1	July 12	84.7	–
	2	July 14	130.0	90.6
	1	July 20	92.9	99.5
Mean ± standard deviation			89.1 ± 14.7	88.3 ± 8.9

men containers, was fortified at 1, 10, 50 and 100 μg kg^{-1} by the addition of diclofop acid (0.25, 2.5, 12.5 and 25 μg, respectively) in 2.5 ml of methanol. The fortified samples were stored at $-10°C$ for a minimum of 24 h prior to extraction. Two replicate samples were analyzed at each fortification level using the non-hydrolytic extraction, whereas four at each level were analyzed using the hydrolytic procedure.

RESULTS AND DISCUSSION

In plants, conjugation of acidic herbicides containing the carboxyl moiety generally involves the formation of an amido linkage by reaction with amino acids/proteins and/or an ester linkage by reaction with sugars, and either type of conjugate could also be expected in exposed animals. Both linkages are susceptible to acid or base hydrolysis and thus, in previous studies [11–13], a hydrolytic step has been part of the analytical method to ensure more accurate quantitation of the parent herbicide.

In the present study, ten of the 24-h urine samples collected from the two "non-applicator" volunteers were analyzed. Half of the samples analysed were collected (June 6–16, 1988) during the normal period of herbicide application on the Canadian prairies with the remainder being collected (July 10–20) following the normal spring spraying season. Background interferences at the retention time for diclofop-methyl for these samples were 0.1 ± 0.1 μg kg^{-1} for both the hydrolytic and non-hydrolyitc methods, and were not significantly greater for urine samples collected during the normal period of herbicide application.

Observed background interferences permitted a limit of quantification of 1.0 μg kg^{-1}. Similar recoveries of diclofop from fortified urine were obtained for both the hydrolytic and non-hydrolytic methods (Table I), and these exceeded 85% (Fig. 2). However, when urine from the exposed applicator was subjected to alkaline hydrolysis (1 *M* NaOH), the concentration of diclofop detected was generally double that from non-hydrolyzed samples (Table II). This indicates that approximately half of the diclofop present in the urine of the exposed applicator was in a conjugated form that would only have been detected after hydrolysis. Consequently, if the hydrolysis had been ommitted, the absorbed dose or amount of diclofop excreted in the urine would have been underestimated by about half. The hydrolytic method had the additional advantage that emulsion problems associated with the methylene chloride extraction were essentially eliminated.

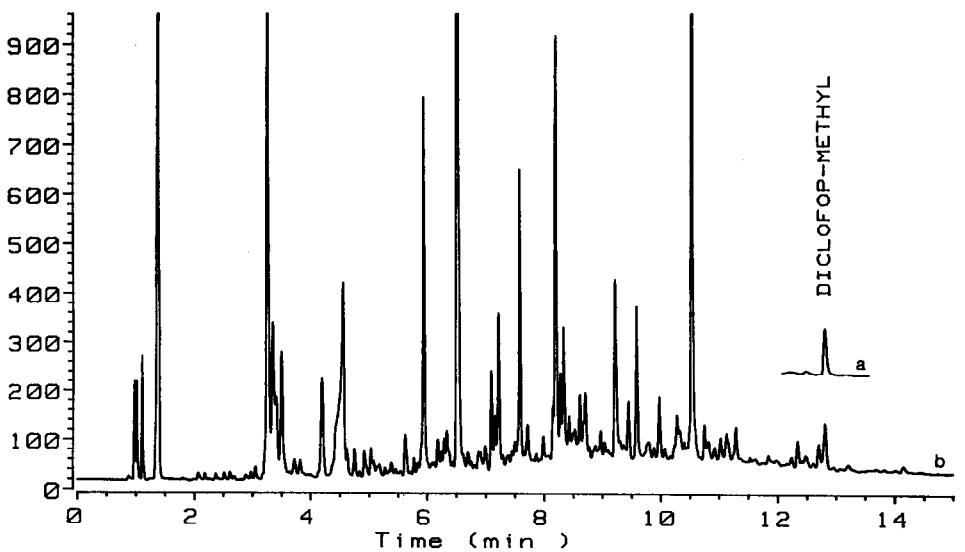

Fig. 2. Gas chromatographic analysis of diclofop residues in urine. Chromatogram a: diclofop-methyl standard (0.2 ng) equivalent to 1 μg kg^{-1}; chromatogram b: recovery (92.9%) of diclofop from urine (volunteer 1; July 20) fortified at 1 μg kg^{-1}.

TABLE II

DICLOFOP CONCENTRATIONS ($\mu g\ l^{-1}$ ACID EQUIVALENT) DETERMINED WITH AND WITHOUT HYDROLYSIS OF URINE SAMPLES COLLECTED FROM THE EXPOSED APPLICATOR

Urine sample	Date	24-h void volume (ml)	Diclofop concentration ($\mu g\ l^{-1}$) in the urine[a]		Total amount (μg) of diclofop excreted in the urine per day	
			With hydrolysis	Without hydrolysis	With hydrolysis	Without hydrolysis
Pre-spraying	June 3	–	–	0.04	–	–
Day of spraying	3	491	0.6	0.4	0.3	0.2
Day 1	4	746	5.7	3.1	4.3	2.3
Day 2	5	948	9.9	5.4	9.4	5.1
Day 3	6	1148	5.5	3.0	6.3	3.4
Day 4	7	1402	7.5	2.7	10.5	3.8
Day 5	8	1260	6.6	2.7	8.3	3.4
Day 6	9	1608	4.4	2.2	7.1	3.5
Day 7	10	961	5.7	4.0	5.5	2.9
Day 8	11	1622	3.1	1.8	5.0	2.9
Day 9	12	893	3.9	2.7	3.5	2.4
Day 10	13	1258	3.9	2.0	4.9	2.5
Cumulated amount					65.1	33.3

[a] Residues are uncorrected for recoveries.

The urinary residue data (Table II) describe an excretion pattern in which the concentration of diclofop in the urine reached a maximum 2 to 4 days after exposure and then continually decreased through to day 10. Similar urinary excretion patterns following dermal exposure have been reported for other acidic herbicides. For example, maximum urinary concentrations have been detected at 1 to 2 days following applicator exposure to MCPA [17] and at 3 days for 2,4-D [18]. However, in contrast to the present study where the urinary concentration of diclofop remained well above background at 10 days after exposure, concentrations of MCPA [17] and 2,4-D [18] returned to background levels 5 and 8 days, respectively, after exposure.

The total amount of diclofop applied by the farmer in the present study was 22.7 kg (a.e.). Cumulative urinary excretion of diclofop (a.e.) following this exposure was 65 μg 10 days after exposure, however, since diclofop residues in the urine remained substantially above background levels even at 10 days after the single exposure (Table II), this represents an underestimation.

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