

The pronounced effect of phosphoric acid treatment of the borosilicate glass derivatization tubes is interesting in light of the observation by Sprankle et al. (1975) that increasing soil phosphate concentrations decreased the amount of GLYPH that adsorbed to the soil. This is consistent with recent observations in this laboratory that indicate that acidic phosphate buffers are reasonably efficient as extraction solvents for removing GLYPH from soil (Moye, 1983).

Work is continuing in this laboratory to employ the presently described derivatization method for the residue analysis of GLYPH on fruits, vegetables, and soil.

Registry No. Glyphosate, 1071-83-6; (aminomethyl)-phosphonic acid, 1066-51-9; *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide, 77377-52-7.

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High-Performance Liquid Chromatographic Determination of Bromoxynil Octanoate and Metribuzin in Runoff Water from Wheat Fields

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A method for the direct determination of the herbicides metribuzin and bromoxynil octanoate and their metabolites DADK and bromoxynil by high-performance liquid chromatography (HPLC) was developed. Aqueous samples were frozen to simulate storage of field samples, thawed in a microwave oven to minimize time of exposure to room temperatures, acidified to pH 3.3, and extracted with dichloromethane/ acetonitrile. Compounds were separated by HPLC on an octadecyl reverse-phase column with a water/methanol gradient and determined with a variable-wavelength ultraviolet detector. High-density polyethylene storage containers adsorbed 50-80% of the bromoxynil octanoate from solution, but satisfactory recoveries were obtained by extracting the freshly emptied containers with dichloromethane. In the range of 20-200 ppb, recoveries averaged 83-96%. At 25 ppb standard deviations were 4.8% for metribuzin, 4.0% for DADK, 6.2% for bromoxynil octanoate, and 1.4% for bromoxynil. Almost complete loss of bromoxynil octanoate occurred at room temperatures (20-25 °C) within 72 h, although no losses of the other three compounds were observed. Samples could be stored safely at -15 °C up to 300 days.

Wheatlands in the Pacific Northwest suffer severe runoff and soil erosion during the winter, resulting from a combination of rain or snow melt on saturated soils, steep slopes, and management that leaves little surface cover for soil protection. Adoption of conservation (reduced) tillage systems can cut soil losses from 25 tons acre⁻¹ (56 Mg ha⁻¹) to 5 tons acre⁻¹ (11 Mg ha⁻¹) or less on the steeply hilled Palouse winter wheat area of Washington, Idaho, and Oregon (Oldenstadt et al., 1982). In this region, herbicides are used extensively for weed control in wheat grown under conventional or reduced tillage. Although most of these chemicals are applied to winter wheat fields in the spring,

there is a potential use for, and at times a need for, fall application prior to the winter runoff season.

Two herbicides that have potential use for fall application in wheat are metribuzin (Sencor, Lexan) and bromoxynil octanoate (Brominal). Both chemicals have relatively short half-lives under spring and summer conditions (Stewart et al., 1975). However, little is known about the persistence and runoff of metribuzin and bromoxynil octanoate after fall or winter application to winter wheat. It is known that cold conditions can increase herbicide persistence dramatically (Hörmann et al., 1979). Furthermore, prolonged frozen field conditions occurring shortly after an herbicide treatment followed by a heavy runoff event potentially could lead to high herbicide content in runoff water weeks after the application.

Recently several methods for the determination of bromoxynil octanoate in wheat plants (Cessna, 1980), wheat products (Lawrence et al., 1980), and soils (Smith, 1980) have been published as have methods for the determination of metribuzin in soils (Vickery et al., 1980),

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grains, plant material, and water (Jarczyk, 1978). None of these methods, however, appears suitable for the simultaneous determination of the two compounds and their metabolites. The purpose of the present work was to develop a liquid chromatographic (HPLC) method for the simultaneous extraction and determination of bromoxynil octanoate and metribuzin and their metabolites, bromoxynil and DADK, in runoff waters.

MATERIALS AND METHODS

Reagents. HPLC-grade dichloromethane, methanol, and water were from MCB, a division of EM Industries, Inc. Water (purified) for recovery and storage experiments was prepared from tap water by using a Culligan Aqua-Clear reverse osmosis unit. Acetonitrile was redistilled reagent-grade, Mallinkrodt. Analytical DADK (desaminodiketometribuzin) and metribuzin [4-amino-6-*tert*-butyl-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] were from the Mobay Chemical Corp, Agricultural Chemical Division, Kansas City, MO. Their respective purities were >98% and 99.6%. Bromoxynil (2,6-dibromo-4-cyanophenol) and bromoxynil octanoate (2,6-dibromo-4-cyanophenyl octanoate) were from Union Carbide Corp., Raleigh, NC (Amchem Products, Inc., Amberly, PA). Their purities were >98%. Gallon (3.78 L) and half-gallon (1.89 L) high-density polyethylene plastic jugs (milk bottles) were obtained from the Georgia Pacific Corp., Tacoma, WA. Samples of runoff water containing soil sediment were collected during the winter of 1980–1981 from field plots established at the Palouse Conservation Field Station, 3 km northwest of Pullman, WA. The winter wheat plots, except check and continuous fallow plots, received surface applications of metribuzin and bromoxynil in the fall and early winter, respectively, and were managed under various crop rotations typical of the area. Samples were taken from collection tanks at the base of the plots, within 24 h after the plots had ceased to run, and were maintained frozen in high-density polyethylene jugs at -15°C until thawed for analysis.

Sample Preparation and Extraction. Samples were thawed to room temperature in a microwave oven using the defrost cycle (Note: Some degree of care must be exercised in thawing the samples because after all the ice has melted the water temperature can rise to $30\text{--}35^{\circ}\text{C}$ within 10 min if the microwave thaw cycle is not terminated.) A 1-L aliquot of the sample was transferred to a 2-L separatory funnel and acidified with 1 mL of glacial acetic acid. Acetonitrile (350 mL) and dichloromethane (200 mL) were added. The funnel was shaken 2 min with frequent venting and the mixture allowed to separate into two layers. If the emulsion did not separate within 5–10 min, it was broken by centrifugation at $850g$ in 250-mL glass centrifuge bottles. The bottom layer was drawn off and retained. The upper, aqueous layer was reextracted with a mixture containing 200 mL of acetonitrile and 200 mL of dichloromethane and a third time with 300 mL of dichloromethane. In the case of samples that had been stored in polyethylene bottles, the freshly emptied bottle also was extracted 4 times with 100-mL portions of dichloromethane, giving a fourth extract. A proportional part of the dichloromethane extract representing 1 L of the original water sample was retained for analysis and the remainder discarded (Volume of dichloromethane retained = $1\text{ L} \times \text{total volume (L) of dichloromethane extract} / \text{volume (L) of sample originally in the jug}$).

After the extracts were dried with anhydrous sodium sulfate, they were filtered through anhydrous sodium sulfate on folded filter paper. The flasks were rinsed 3 times with 5-mL portions of dichloromethane and the filter

paper was washed 3 times with 5-mL portions of dichloromethane. A keeper (0.2 mL containing 1 mg of decanol/mL of methanol) was added to the first extract. The extracts were combined and concentrated with a rotary evaporator ($<34^{\circ}\text{C}$ and 95 kPa) to a final volume of 1 mL. Samples were made to volume with methanol in 10-mL volumetric flasks and then were filtered by using a Swinny adapter and syringe through $1\text{ }\mu\text{m}$ porosity Millipore, Fluoropore, filters into 2-mL vials and capped. Vials were stored under refrigeration until analyzed.

Apparatus and HPLC Analytical Procedure. The high-performance liquid chromatograph was a Hewlett-Packard (HP), Model 1084B, equipped with a variable-wavelength detector and an HP (catalog no. 79916B) RP-18, reverse-phase, $200 \times 4.6\text{ mm i.d.}$, $10\text{ }\mu\text{m}$ analytical column. The mobile phase used for gradient elution of the compounds consisted of a mixture of water (A) and methanol (B) that contained 0.5 mL of glacial acetic acid/L. The chromatographic parameters were (1) flow 2 mL/min, (2) attenuation 3, (3) slope sensitivity 0.05, (4) solvent heater A 80°C , (5) solvent heater B 40°C , (6) ambient oven temperature, (7) sample volume 100 mL, and (8) initial wavelength 262 nm (for DADK). The solvent was programmed to run from 28% B to 37% B in 15 min, to reach 39% B at 20 min, to hold until 22.5 min, and then to rise to 69% B at 23.5 min and hold for 30 min. Residual organic compounds were removed from the column by eluting with 100% methanol for 5 min. The detector was programmed for (1) 298 nm for metribuzin at 13 min, (2) 260 nm for bromoxynil at 18.5 min, and (3) 240 nm for bromoxynil octanoate at 44 min. Wavelength settings for analysis of the four compounds were chosen on the basis of UV spectra obtained with the variable-wavelength detector built into the chromatograph.

Effect of Temperature, Storage Time, and Sodium Azide on Herbicide Stability. Known amounts of the two herbicides and their metabolites were added to 1-L portions of purified tap water or runoff water from the check plots in 3.78-L brown-glass reagent and high-density polyethylene bottles. The bottles were capped, shaken vigorously, and stored in the dark for various periods of time. Some samples also were fortified with 100 mg/L (0.01%) sodium azide. At the end of the storage period, samples were extracted as outlined under Sample Preparation and Extraction.

Recovery of Compounds from High-Density Polyethylene Bottles. Known amounts of the two herbicides and their metabolites were added to 3.78-L glass reagent and high-density polyethylene bottles containing 1 L of purified water. Bottles were capped, shaken vigorously, and frozen. After frozen storage of up to 4 weeks at -15°C , the samples were thawed and the contents extracted as outlined under Sample Preparation and Extraction. Extracts from bottles and water were analyzed separately.

Effect of Prior Acidification of Water on Recovery of Herbicides. Liter portions of purified water (pH 7.1) were fortified with the four compounds together and stored for up to 4 weeks in glass and polyethylene bottles at -15°C . Samples were randomly assigned to two groups. Half of the bottles in each group were acidified to pH 3.3 with glacial acetic acid before being extracted. The other bottles were extracted without pH adjustment. Dichloromethane extracts were combined with water extracts during rotary evaporation.

Stability of Herbicides during Frozen Storage in High-Density Polyethylene Bottles. Polyethylene bottles (1.89 L) containing 1-L portions of purified water were fortified with 30 or 150 μg (ppb) of the four com-

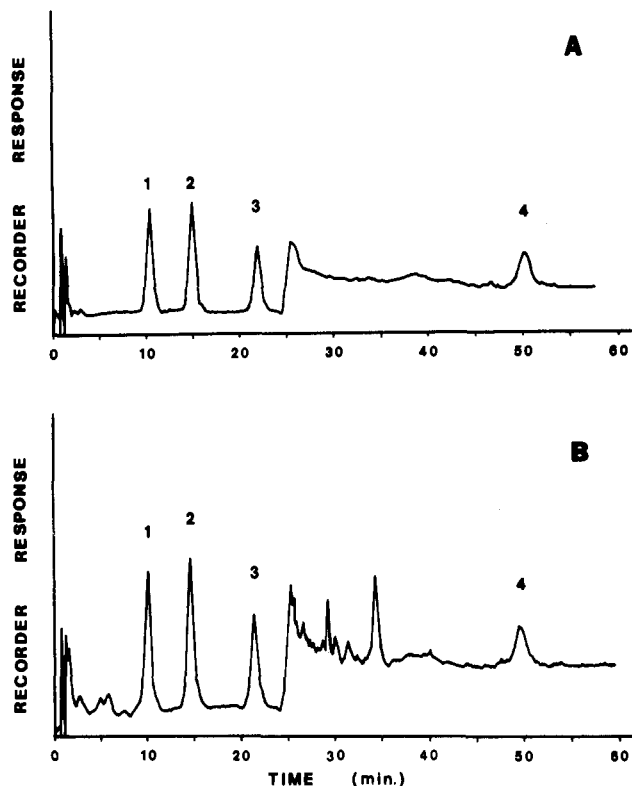


Figure 1. HPLC chromatograms using an RP-18 reverse-phase column, a variable-wavelength detector, and a water/methanol gradient (see the text for details). (A) Analytical standards (0.02 μg injected). (B) Standard after extraction from purified water prepared by reverse osmosis of tap water (original concentration 30 ppb). 1 = desaminodiketometribuzin (DADK); 2 = metribuzin; 3 = bromoxynil; 4 = bromoxynil octanoate. The base-line change at 24 min was caused by an increase in methanol concentration from 39 to 69%.

pounds together, shaken vigorously, frozen immediately, and then stored at -15°C for up to 10 months. Samples were thawed to room temperature in the microwave oven ($1/2$ h) and acidified, and extraction was completed within 3 h to minimize exposure to room temperature. Dichloromethane rinses from the bottles and their respective water extracts were combined during rotary evaporation.

Determination of Metribuzin, DADK, Bromoxynil Octanoate, and Bromoxynil in Simulated Runoff Samples. Known amounts of the four compounds (20–200 ppb) were added to 1.89-L polyethylene bottles containing 1-L portions of runoff waters from continuous-tilled fallow plots. Recovery experiments were run with all four compounds together, as well as in the pairs, DADK/bromoxynil and metribuzin/bromoxynil octanoate. Samples were mixed thoroughly, frozen, and stored at -15°C for periods up to 60 days or more. The samples were thawed and then extracted and analyzed as outlined under Sample Preparation and Extraction and under Apparatus and HPLC Analytical Procedure.

RESULTS AND DISCUSSION

HPLC Conditions. Bromoxynil is an acidic phenol with a $\text{p}K_a$ of about 4.2 (Cessna and Grover, 1978). Acidification of the solvents with glacial acetic acid (0.5 mL/L) shifted the elution time of bromoxynil from the solvent front to after elution of metribuzin (Figure 1A). Retention times of DADK, metribuzin, and bromoxynil octanoate were not altered appreciably. Bromoxynil octanoate was retained much more strongly than the other three compounds and required high concentrations of methanol (exceeding 68%) in order to elute the octanoate

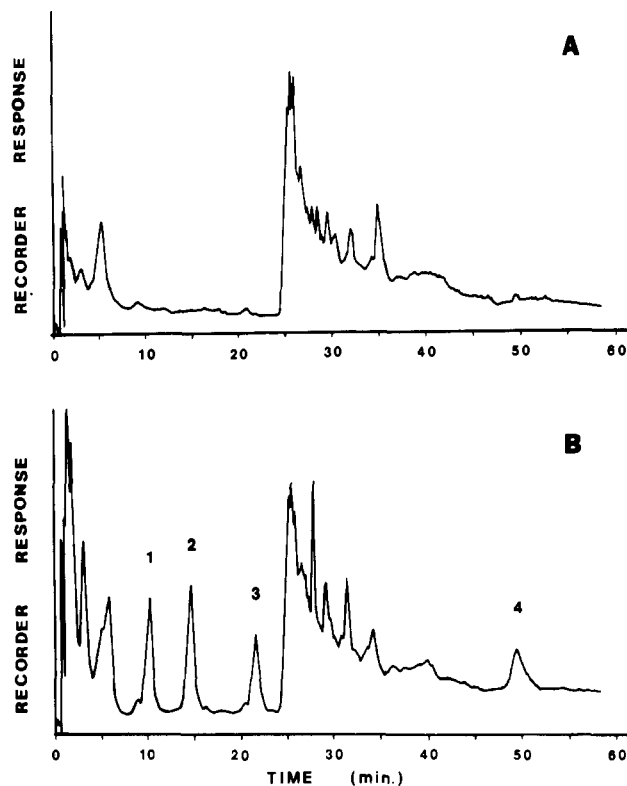


Figure 2. (A) HPLC chromatogram of the extract of runoff water from continuous-tilled fallow plot (check). (B) Chromatogram of extract of runoff water from continuous-tilled fallow plot fortified with standards (30 ppb). Conditions and labeling are the same as in the legend of Figure 1.

Table I. Recovery of Metribuzin, DADK, Bromoxynil Octanoate, and Bromoxynil from Continuous-Tilled Fallow Plot Runoff Water

ppb added	recovery, % ^a			
	metribuzin	DADK	bromoxynil octanoate	bromoxynil
20	87.4	93.2	100.2	101.0
25	83.4	95.0	79.2	102.8
50	86.4	85.4	83.5	89.7
100	89.9	89.6	75.4	94.6
200	84.2	87.6	78.2	88.4
av	86.0	90.6	82.9	96.0

^a Average values from two independent 1-L samples, except averages are from three independent samples at 25 ppb.

from the RP-18 column in less than 30 min (Figure 1A).

A number of extraneous UV-absorbing compounds were extracted from samples made by fortifying water purified by reverse osmosis of tap water (Figure 1B). The number and quantity of UV absorbing compounds were greater in samples prepared from runoff water from continuous-tilled fallow plots (Figure 2A). A low methanol concentration (69%) with an abnormally long run time was needed to resolve bromoxynil octanoate from the large quantities of interfering compounds that were present in the yellowish extracts obtained from heavily sedimented, late-fall runoff waters.

Recovery of Metribuzin, DADK, Bromoxynil Octanoate, and Bromoxynil from Runoff Water. Recovery data for samples prepared by fortifying 1-L portions of runoff water samples from continuous-tilled fallow plots with 20–200 ppb of the four compounds together are shown in Table I. Recoveries ranged from 75 to 103%. At 25 ppb the mean recoveries and their standard deviations were (1) $83.4 \pm 4.8\%$ for metribuzin, (2) $95.0 \pm 4.0\%$ for

Table II. Effect of Adjusting pH to 3.3 with Acetic Acid on Recovery of Herbicides from Water^{a, b}

ppb added	% recovery							
	pH 7.1				pH 3.3			
	metribuzin	DADK	bromoxynil octanoate	bromoxynil	metribuzin	DADK	bromoxynil octanoate	bromoxynil
30	84.3	88.0	84.2	45.8	75.0	86.8	95.1	87.1
90	87.8	87.9	89.2	37.5	85.6	93.1	86.4	92.7
300	91.8	88.5	76.3	26.6	85.2	83.6	72.0	85.7

^a Samples in 1 L of water prepared by reverse osmosis of tap water. ^b Recovery data average of four samples: two stored in plastic bottles; two stored in glass bottles.

DADK, (3) $79.2 \pm 6.2\%$ for bromoxynil octanoate, and (4) $102.8 \pm 1.4\%$ for bromoxynil. A typical chromatogram obtained during analysis of the runoff water that had been fortified with the four compounds (30 ppb) is shown in Figure 2B. Below 20 ppb recoveries were erratic. At 10 ppb, mean recoveries and standard deviations were (1) $64.9 \pm 22.4\%$ for metribuzin, (2) $73.1 \pm 28.3\%$ for DADK, (3) $74.0 \pm 6.4\%$ for bromoxynil octanoate, and (4) $80.6 \pm 31.6\%$ for bromoxynil. Determination of the four compounds was very difficult at 5 ppb. The problem was particularly severe with bromoxynil and bromoxynil octanoate, whose peaks were flatter and broader than the others because of the longer retention times. At 5 ppb, which we consider to be the practical limit of detection, recoveries ranged from 45 to 148%.

A series of samples fortified with pairs of the compounds, metribuzin plus bromoxynil octanoate and DADK plus bromoxynil, also were prepared in fallow plot runoff water. These samples were carried through the entire analytical procedure including freezing and thawing. No breakdown or contamination from metribuzin, DADK, or bromoxynil was detected. Samples fortified with bromoxynil octanoate yielded an average of 4.1% bromoxynil (data not shown). Half of the extraneous bromoxynil (2%) was present as a contaminant in the original bromoxynil octanoate analytical standard. Data reported in this paper have not been corrected for the low level of bromoxynil introduced in the bromoxynil octanoate.

The sampling, storage, and analytical procedures also were utilized in preliminary field tests. Two chromatograms obtained from runoff waters collected at the base of field plots treated with metribuzin and bromoxynil octanoate are shown in Figure 3. The sample in Figure 3A was collected in a minor runoff event that occurred 65 days after the metribuzin and 6 days after the bromoxynil octanoate were applied to the plots. The sample in Figure 3B was collected in a major runoff event 128 days after the metribuzin and 69 days after the bromoxynil octanoate were applied to the plots.

Resolution of metribuzin, DADK, and bromoxynil was good in both types of samples. Resolution of the zone in the chromatogram corresponding to the retention time for bromoxynil octanoate also was good in Figure 3B. In Figure 3A the retention time for bromoxynil octanoate (49 min) corresponds to the bottom of the valley in Figure 3A. We think that the large amounts of interfering compounds contained in the yellowish extracts from the late fall runoff events (like the one in Figure 3A) come from partially decomposed plant materials in the runoff sediments. Lower methanol concentrations and longer elution periods than those used in developing the method can be used to completely resolve bromoxynil octanoate in these highly pigmented samples. It is also interesting to note that neither sample contained measurable concentrations of bromoxynil octanoate and that the sample collected 6 days after the bromoxynil octanoate treatment contained a relatively high concentration of bromoxynil.

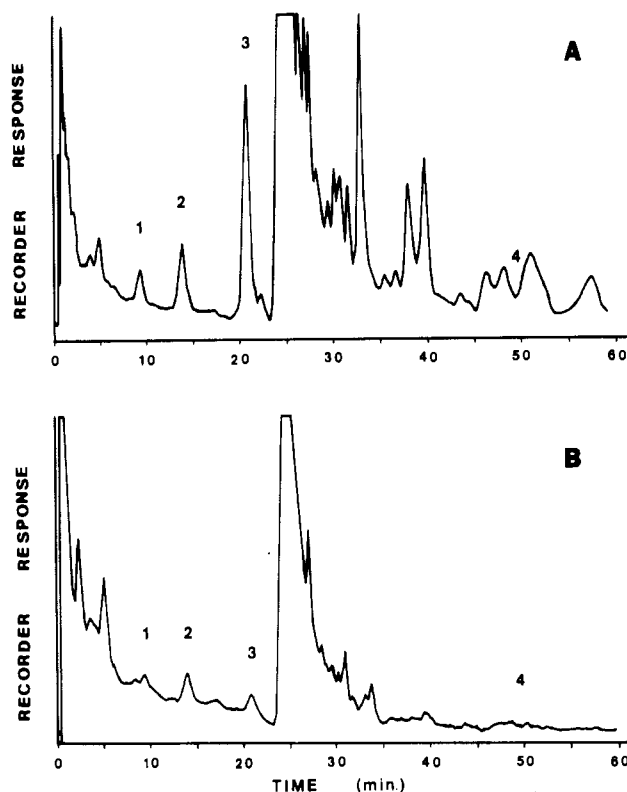


Figure 3. (A) HPLC chromatogram of the extract of runoff water from a light runoff event occurring 65 days after metribuzin and 6 days after bromoxynil octanoate treatment. Concentrations found: 1 = 5.7 ppb; 2 = 14.1 ppb; 3 = 73.8 ppb; 4 = 3.1 ppb. (B) HPLC chromatogram of the extract of runoff water from a heavy runoff event 128 days after metribuzin and 69 days after bromoxynil octanoate treatment. Concentrations found: 1 = 3.3 ppb; 2 = 6.3 ppb; 3 = 7.1 ppb; 4 = 0 ppb. Conditions and labeling are the same as in the legend of Figure 1.

Extraction of Herbicides. Inclusion of acetonitrile and acetic acid along with dichloromethane in the extraction solvent is needed to ensure adequate recovery of DADK and bromoxynil from water. Incorporation of dichloromethane brought recovery of DADK to satisfactory levels, but recoveries of bromoxynil from purified water (pH 7.1) remained low and erratic. Acidification of the water to pH 3.3 with glacial acetic acid depressed ionization of bromoxynil's phenolic group and increased recoveries to acceptable levels (Table II). Acidification did not affect recoveries of DADK, metribuzin, and bromoxynil octanoate.

Effect of Storage Time and Temperature on Recovery of Herbicides from Water. Although samples containing bromoxynil octanoate could be stored in the frozen state for prolonged periods (see below), the compound degraded in aqueous solution. As shown in Table III, approximately 42% of the bromoxynil octanoate was lost from water samples containing 30 ppb of bromoxynil octanoate during room temperature storage for 24 h, and

Table III. Effect of Room Temperature Storage and Addition of Sodium Azide on Recovery of Herbicides from Water^a

ppb added	room temp storage time, h	% sodium azide added	no. of samples ^b	% recovered			
				M ^c	DADK	BO ^c	B ^c
30	0	0	3	87.0	94.3	89.7	89.0
30	24	0	3	70.8	82.6	58.2	111.4
25	72	0	2	85.6	84.0	11.6	139.9
30	24	0.01	3	82.5	77.7	35.7	128.1

^a Samples in 1 L of water: one sample in each set prepared from runoff water; additional samples prepared from purified tap waters. ^b Number of samples. ^c M = metribuzin; BO = bromoxynil octanoate; B = bromoxynil.

Table IV. Percentage Recovery of Herbicides from Water after Storage in Glass Bottles for up to Four Weeks at -15 °C^a

ppb added	no. of samples ^b	bromoxynil			
		metribuzin	DADK	octanoate	bromoxynil
30	2	68.9 ^c	84.8	91.0	87.2
90	2	82.6	95.2	86.2	92.7
300	2	89.4	87.0	73.0	91.8

^a Samples in 1 L of water prepared by reverse osmosis of tap water. ^b Number of samples. ^c Metribuzin recoveries at 30 ppb averaged 59.6 and 78.2%.

Table V. Dichloromethane Recovery of Bromoxynil Octanoate Adsorbed to Walls of High-Density Polyethylene Bottle during Frozen Storage

ppb added	% bromoxynil octanoate recovered ^{a, b}		
	aqueous fraction	interior surface of freshly emptied bottle	total
30	10.6	83.1	93.7
90	8.2	64.4	72.6
150	8.0	71.2	79.2
300	29.9	50.5	80.4

^a Samples in 1 L of purified water prepared by reverse osmosis of tap water. ^b Average recoveries from duplicate samples.

the loss of bromoxynil octanoate was accompanied by a corresponding increase in bromoxynil. Within 72 h 88% of the bromoxynil octanoate had been degraded to bromoxynil. Instead of preventing degradation, addition of sodium azide, a potent inhibitor of growth and metabolism in microorganisms, resulted in a more rapid degradation of the octanoate.

The rapid rate of bromoxynil octanoate degradation that we observed during room temperature storage of the aqueous samples is in agreement with the findings of

Collins (1973), who reported the complete degradation of bromoxynil octanoate within 3 days at 15 °C during perfusion tests using six soils. The rapid rate of bromoxynil octanoate degradation in water and the even more rapid rate of degradation in the presence of 0.01% (0.0015 M) sodium azide could be explained most easily by a base-enhanced hydrolysis mechanism. Microorganismal degradation of bromoxynil octanoate can occur, but this explanation seems unlikely because the inclusion of sodium azide did not deter the degradation of the octanoate in our samples. An explanation based on photodegradation also appears unlikely because the storage tests were conducted in brown-glass bottles located in the corner of a laboratory fume hood.

Noticeable degradation of the herbicides in aqueous solutions containing from 30 to 300 ppb of all four compounds was prevented for up to several weeks by storing the samples at -15 °C (Table IV). Recoveries from samples stored frozen in glass bottles were satisfactory. When high-density polyethylene bottles were used as the storage containers, a large amount of the bromoxynil octanoate was adsorbed on the inside surface of the polyethylene bottle (Table V). Extraction of the freshly emptied plastic bottle with four 100-mL portions of dichloromethane yielded an additional 50–83% of the bromoxynil octanoate and brought the average total recovery of bromoxynil octanoate to 81% over the range of 30–300 ppb.

Results of the long-term frozen storage stability test are shown in Table VI. Herbicide recoveries from samples stored at -15 °C remained high throughout the 300-day study. The overall results indicate that water samples containing metribuzin, DADK, bromoxynil, and bromoxynil octanoate can be stored for at least 300 days at -15 °C without undergoing serious degradation.

Adsorption Losses. Loss of trace components by adsorption during sample preparation and storage has been established previously by others. Several authors have reported loss of herbicides by adsorption to glass and plastics. Strachan and Hess (1982) found that in aqueous solution five dinitroaniline herbicides were adsorbed onto glass and reached equilibrium in a matter of minutes. Connick and Simoneaux (1982) reported that dichlobenil was adsorbed on their polyethylene bottle cap liners and on cellulose acetate-nitrate, 0.45 μm, Millipore filters. Sharom and Solomon (1981) reported data on the adsorption of seven herbicides, including metribuzin, on glass and poly(vinyl chloride). Metribuzin was slightly adsorbed on glass and up to 45% of the metribuzin in a solution containing 1350 ppm could be adsorbed on poly(vinyl chloride). The authors reported that permethrin was adsorbed strongly on polyethylene and Teflon, but data were not given for metribuzin on polyethylene and Teflon. Our data indicate that neither metribuzin nor DADK and bromoxynil are adsorbed significantly by glass or high-

Table VI. Average Percent Recoveries of Herbicides after Frozen Storage at -15 °C in Polyethylene Bottles for Up to 300 Days

days of storage	ppb added ^{a, b}							
	30				150			
	metribuzin	DADK	bromoxynil octanoate	bromoxynil	metribuzin	DADK	bromoxynil octanoate	bromoxynil
0	81.0	88.7	99.2	89.0	82.3	91.2	79.1	91.6
69	85.7	84.8	74.0	89.2	82.8	83.0	86.2	90.1
180	90.0 ^c	91.4 ^c	86.0 ^c	92.6 ^c	89.3	91.0	86.8	91.3
210	85.4	86.2	82.9 ^c	91.6				
300	81.0	86.6	82.8	89.5	90.1 ^c	85.8 ^c	67.9 ^c	87.6 ^c

^a Samples in 1 L of purified water prepared by reverse osmosis of tap water. ^b Average recoveries from duplicate samples. ^c Single sample.

density polyethylene from solutions containing up to 300 ppb of the compounds. Our data also indicate that bromoxynil octanoate is not adsorbed appreciably by glass, although up to 80% of bromoxynil octanoate in aqueous solution can be adsorbed on polyethylene.

Comments. In summary, we have developed an HPLC method for the direct determination of metribuzin and bromoxynil octanoate and their respective metabolites, DADK and bromoxynil, in runoff water from wheat fields when the water contained 5–200 ppb ($\mu\text{g/L}$) of the compounds. The compounds are partitioned into dichloromethane/acetonitrile after acidifying the water sample with acetic acid. After the extracts are reduced in volume and dried over anhydrous sodium sulfate, the compounds are separated on a reverse-phase octadecyl column by using an acidified water/methanol gradient and determined with a variable-wavelength detector.

The crucial points in utilizing our method are minimization of exposure of samples containing bromoxynil octanoate to room or elevated temperatures, freezing of field samples as soon as possible, and making sure that the pH of the samples is low enough to inhibit ionization of the free phenolic group on bromoxynil during extraction. If samples are stored in polyethylene and probably other plastic containers, a rinsing step with an organic solvent like dichloromethane must be included to recover bromoxynil octanoate adsorbed on the interior surfaces of the plastic containers.

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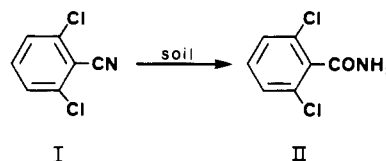
Simultaneous Determination of 2,6-Dichlorobenzonitrile and 2,6-Dichlorobenzamide in Aqueous Samples by a Direct High-Performance Liquid Chromatographic Method

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The herbicide dichlobenil (2,6-dichlorobenzonitrile) and its degradation product in soil, 2,6-dichlorobenzamide, have been directly and rapidly determined in aqueous samples by high-performance liquid chromatography (HPLC). Using a radial compression C_{18} column, 50% CH_3CN –50% H_2O mobile phase (2.0 mL/min), 100- μL sample injection, and UV detector at 205 nm, the method described gave a detection limit of 0.01 ppm for each compound without a preliminary extraction or concentration step. This method should be useful in a variety of applications involving dichlobenil formulations and their fate in soil or water over a wide concentration range.

Dichlobenil (2,6-dichlorobenzonitrile) (I) (Scheme I) is a broad-spectrum, somewhat volatile, herbicide that is tolerated well by many established crops. It can be used for total weed control in noncrop situations and is also effective in aquatic weed control (Weed Science Society of America, 1979). Dichlobenil is degraded by soil and hydrosol microorganisms to 2,6-dichlorobenzamide (II) (Beynon and Wright, 1972; Montgomery et al., 1972;

Scheme I



Verloop, 1972). Amide residues in soil are sometimes present in greater amounts than the parent dichlobenil (Beynon and Wright, 1968; Khan and Miller, 1982).

Residue determinations of I and II usually employ an extraction step, column chromatography for extract

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