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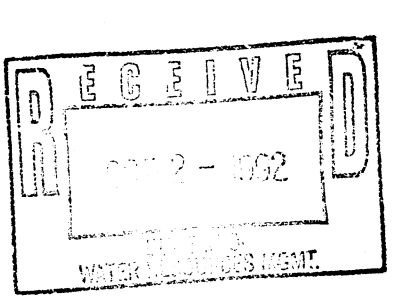
ANALYTICAL DETERMINATION OF PESTICIDE METABOLITES AND CARRIER CHEMICALS IN WISCONSIN WELLS



FINAL REPORT

Analytical Determination of Pesticide Metabolites and Carrier Chemicals in Wisconsin Wells

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Summary of Accomplishments

Overall, the project was very successful. Much useful experience was gained analyzing pesticides and pesticide metabolites using high performance liquid chromatography (HPLC). Valuable experience was also gained using solid phase extraction as a concentration technique. This technique promises to be very valuable in the future. Several pesticide metabolites were identified that were not identified by the State Laboratory of Hygiene previously. Training was provided to a scientist from Israel who spent nearly a year on the project while he was on scientific sabbatical; in turn, the scientist provided valuable input to the project at no cost to the state since his salary was covered by his employer in Israel.

Measurements were made of atrazine, alachlor, cyanazine and metribuzin. Early in the study, upon the request of the Wisconsin Department of Natural Resources personnel, it was decided to focus much of the analytical work on metribuzin. Metribuzin is a widely used pesticide, particularly in sandy areas of the state where contamination of groundwater from surface application of pesticides is a problem. A method to measure metribuzin and its likely metabolites was developed and it proved to be better than any reported in the literature when metribuzin and all metabolites were considered. The method employed solid phase extraction and high performance liquid chromatography. The method was used to test groundwater samples, collected by the Wisconsin Department of Agriculture, Trade and Consumer Protection, for metribuzin and metabolites. Analysis of

these samples showed that all of the key metabolites of metribuzin can exist in groundwater, sometimes at concentrations in excess of the parent compound. Besides metribuzin, the method was used for measuring atrazine and metabolites, alachlor and metabolites and cyanazine and metabolites. Thus, it showed promise as a multiresidue technique. The work culminated in a technical paper submitted for publication to the journal Water Research (copy attached).

Last, mention should be made of carrier chemicals used in pesticide formulations. Because this information is proprietary, it is naturally difficult to get information from chemical suppliers on the carrier chemicals they use. A list of fifteen different inert carriers was finally obtained from Ciba-Geigy. Most of these compounds appear to be emulsifying agents. None of the compounds appear to be an environmental hazard, based on our literature assessment of the compounds. The list of carrier chemicals is not included here as Ciba-Geigy requested the list be kept confidential.

Technical Discussion

The progress made in this study was chronicled in the quarterly reports submitted to the Department of Natural Resources. Besides these quarterly reports, weekly minutes that document the progress and decisions made during the study were prepared. These reports are available on request. The summary below shows the course followed in this research project.

Early in the study it was decided that solid phase extraction (SPE) was a promising technique for concentrating

water soluble pesticides (of interest in this study) before analysis by high performance liquid chromatography (HPLC). SPE cartridges had not been used before at the Laboratory of Hygiene, so there was no experienced personnel or the necessary equipment in the laboratory. Thus, some time was invested in studying the literature so as to become familiar with the technique.

Initial testing of the cartridge extraction method was done with homemade apparatus that first had to be developed. This apparatus was sufficient to demonstrate the appropriateness of SPE for herbicide and metabolite analysis. This initial success prompted the acquisition of a vacuum manifold that would allow the simultaneous extraction of several samples. Once the vacuum manifold was obtained, some time was required to optimize the extraction technique. The SPE technique using the new SPE manifold was first employed to analyze alachlor and its metabolites. Table 1 shows the results of alachlor analyses performed using SPE and HPLC. As can be seen from the table, the technique worked quite well. The technique probably is preferable to the previously studied (earlier DNR groundwater grant) gas chromatography technique for alachlor. Both the hydroxy alachlor and alachlor sulfonyl metabolites recovered well with the SPE technique. The hydroxy alachlor metabolite in particular was difficult to measure using traditional gas chromatography.

Despite success with alachlor, metribuzin proved difficult to analyze at first. Metribuzin itself recovered quite well, but none of the three metabolites recovered very well. After much

experimentation, the first breakthrough occurred when it was found that acidification of the water sample with 3.0 grams of glacial acetic acid per liter of sample resulted in excellent recoveries of both metribuzin and deaminated metribuzin (DA). Unfortunately, the addition of acid improved the recoveries of the other two metabolites only slightly (to only 8-15%). Next,

Table 1. Alachlor and Metabolite Recoveries Using Solid Phase Extraction Followed by HPLC/UV Analysis.

Compound	Spike (μg/L)	% Recovery
Alachlor	11.6	105
Alachlor	11.6	117
Alachlor	11.6	97
Alachlor Sulfonyl	9.9	110
Alachlor Sulfonyl	9.9	109
Alachlor Sulfonyl	9.9	108
Alachlor Sulfonyl	9.9	109
Alachlor Sulfonyl	9.9	103
Alachlor Sulfonyl	9.9	105
Alachlor Sulfonyl	9.9	103
Alachlor Sulfonyl	9.9	107
Alachlor Sulfonyl	9.9	106
Hydroxy Alachlor	9.9	107
Hydroxy Alachlor	9.9	88
Hydroxy Alachlor	9.9	94

the amount of cartridge material (solid phase extractant) was increased to one gram. This led to a small improvement in recovery of the diketo- (DK) and the deaminated diketo metribuzin

(DADK) metabolites (the latter increased to about 20%).

At this point an experiment was conducted to determine if addition of sodium chloride (salt) to the sample prior to the solid phase extraction improved recoveries. Salt addition is a common technique used to improve recoveries in more conventional liquid-liquid extraction techniques. The salt addition resulted in recoveries of about 14% for DK and about 30% for DADK when 250 grams of salt were added to the sample before extraction.

Finally, a combination of measures was tried. It was found that by using two 1 gram cartridges in series (instead of a single 1 g cartridge or smaller cartridge), acidifying the sample and adding 150 grams of salt improved recoveries appreciably. Further, after much trial and error, it was found that carefully maintaining the manifold pressure drop at just under 5 inches mercury improved recoveries. Using these measures 10 microgram spikes of DK and DADK could consistently be recovered at about 35% and 62%, respectively. Though these recoveries are not as high as normally desirable, they are higher than previously reported in the literature (5-15%). Nevertheless, because of the relatively low recoveries, at least for DK, results should be considered semi-quantitative.

Even before the SPE technique was optimized, the HPLC technique, using a HP 1090 HPLC with an HP 85B work station, had to be worked out. Alachlor and its metabolites do not have significant UV absorption except in the 210 nanometer region. In this region, many organic compounds have very strong absorptions; hence, minute trace impurities in even HPLC grade eluants can

cause significant interferences. Fortunately, potentially interfering chromatographic peaks in the methanol/water eluant system we used eluted at very different times from any of the alachlor compounds that we studied. The rather noisy baseline at 210 nanometers did, however, result in higher limits of detection for the alachlor group than those obtained for metribuzin. Even so, the method developed here were adequate to recover and measure alachlor and two of its metabolites (alachlor sulfonyl metabolite, and hydroxyalachlor metabolite) at levels of about 1 g/L.

Note that the metabolites of alachlor studied were chosen from a large and often contradictory assortment of possible metabolites reported in the literature. Most are probably not likely to be found in groundwater. A third alachlor metabolite, the alachlor sulfonic acid metabolite, was in fact searched for. It was expected that the technique developed can detect this compound. However, the standard solution of this metabolite deteriorated quickly at room temperature (it apparently degraded in only a few days). Thus, it was deemed not to be a likely metabolite in ground water. This metabolite was not suspected (i.e., no peak at an expected retention time) in any of the actual groundwater samples collected and analyzed during the study. We did, however, find alachlor in some samples. Note also that the amount of alachlor found using our SPE/HPLC method agreed well with results of conventional GC analyses on several samples analyzed by both techniques.

To improve the efficiency of the HPLC part of the analyses,

during the project it was necessary to upgrade the original tape drive of the instrument with a faster 400 kilobyte disk drive. Even with the upgraded disk drive resulted in considerable improvement, it was concluded that the HPLC instrumentation overall was somewhat slow and cumbersome for doing production type work. The machine was purchased in 1984, so technology has changed considerably since then. For example, one timesaving technique is to confirm identifications using a compound's UV spectra as a fingerprint. Though the software was upgraded to allow obtaining UV spectra on-line, the quality of these spectra was too poor to be of much use in confirming detection. Thus, we had to resort to the less satisfactory method of using a second column for confirmation. The aging computer on the instrument used often functioned poorly, slowing the pace of the study considerably. On the other hand our experience is not unlike the experience of other laboratories using HPLC. HPLC appears to be more difficult to maintain and to troubleshoot. Consequently, even with a new instrument, work by HPLC is likely to take longer and be less reproducible than gas chromatography. Gas chromatography is generally preferred, but for some contaminants HPLC is the only viable technique.

In addition to development of methods for recovery and analysis of alachlor and metribuzin and their respective metabolites, analytical methods for cyanazine and its metabolites were investigated. One of the main metabolites of cyanazine is identical to the deisopropyl metabolite of atrazine; the other is an amide derivative. This derivative, as well as the deisopropyl

metabolite and the parent compound, was recovered quite well by the liquid-liquid extraction/gas chromatography technique already well established. Consequently, no further work was done for cyanazine.

An investigation was undertaken to demonstrate that SPE followed by HPLC could be used to replace the liquid/liquid (methylene chloride) extraction methods commonly used for GC analyses of herbicides at the State Laboratory of Hygiene. The SPE/HPLC technique successfully recovered 92% of an alachlor spike, 94% of a metolachlor spike, 109% of a cyanazine spike, 25% of a deethylatrazine spike, and 10% of a deisopropyl atrazine spike. These recoveries are very similar to those obtained using liquid-liquid extraction/GC techniques without the addition of salt to the sample. Methods (using salt with extraction by methylene chloride) developed in the previous grant allow for quantitative recoveries of the two atrazine metabolites as well. The experiments with metribuzin metabolites described above show that, for poorly recovered metribuzin metabolites, salting results in greatly improved recoveries using SPE/HPLC. It appears that SPE/HPLC, combined with salting, is an alternative to the more expensive and environmentally less desirable liquid/liquid extraction. Although the HPLC is more difficult to keep running trouble free, the use of large quantity of solvents in the laboratory is becoming more expensive and more of a safety concern. The ability to do several SPE extractions simultaneously with a commercially available manifold is another plus for SPE. Although alachlor and metribuzin metabolites

require quite polar solvents (such as methanol or ethyl acetate) to elute the materials from the SPE cartridges (whereas the other herbicides in our exploratory experiment were extracted into the less polar methyl tertbutyl ether), further research may show the ability of a single cartridge extraction to be used for the analysis of a broad range of herbicides and many of their metabolites. This could be much cheaper, more environmentally friendly, and, perhaps, less time consuming than the currently used methods.

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METRIBUZIN AND METABOLITES IN WISCONSIN (USA) WELL WATER

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Abstract

Metribuzin is a commonly used pesticide in Wisconsin and elsewhere, although contamination of groundwater by metribuzin has received little attention. A technique using solid phase extraction and high performance liquid chromatography was optimized to allow measurement of metribuzin and its major metabolites-- deaminated metribuzin (DA), diketometribuzin (DK) and deaminated diketometribuzin (DADK). Percent recoveries and precision of metribuzin and DA analyses were good. Recoveries and reproducibility of DADK were acceptable (about 65%). However, the recoveries for DK were low (about 35%; the precision of the analysis was good, however). Overall, accuracy and precision were as good as, or better than, reported in the literature. Metribuzin and metribuzin metabolites were found in most of the groundwater samples analyzed from a number of wells located in sandy soil areas of Wisconsin. Metabolites, which have been rarely reported to occur in water, were found in most of the samples even when the parent compound was absent.
Key Words: pesticides, metribuzin, water quality, groundwater, chromatography, toxic substances, agriculture, Wisconsin, drinking water, health effects.

Introduction

Concern over ground water contamination by the products of environmental degradation of agriculturally applied chemicals has begun to match the concern over contamination by the applied chemicals themselves (U.S. Environmental Protection Agency, 1990 and 1992). With the advent of increasingly sensitive analytical methods, a growing number of agricultural chemicals and their metabolites (degradation or breakdown products) are being detected and measured in groundwater. Indeed, pesticide metabolites are even being included in regulatory standards for pesticides in potable groundwater (Belluck et al. 1991).

One pesticide whose metabolites have received very little attention as groundwater contaminants is metribuzin. Metribuzin [4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] is sold under various

trade names, such as Bay, Lexone, and Sencor, by several manufacturers. The herbicide is known to metabolize in the environment chiefly into three compounds: deaminated metribuzin (DA), diketometribuzin (DK), and deaminated diketometribuzin (DADK) (Thornton and Stanley, 1977; Parker et al., 1983). The generally accepted environmental breakdown scheme for metribuzin is shown in Figure 1.

Metribuzin is widely used as a pre- and post-emergent control for grassy and broadleaf weeds in soybeans and potatoes. For example, about 78% of the Wisconsin acreage used to grow potatoes is treated with metribuzin (Postle, 1991). The use of metribuzin on potatoes is of particular interest, since potatoes are commonly grown in the sandy soils of the lower Wisconsin River valley and the area in central Wisconsin known as the central sands area. These regions, which have shallow water tables, are well known for their susceptibility to groundwater pollution by pesticides. Metribuzin, in fact, has been detected in a number of wells in this area as a result of recent monitoring by the Wisconsin Department of Agriculture, Trade and Consumer Protection (Postle 1991).

Only limited research has been conducted on the environmental fate of metribuzin in groundwater or in surface waters. Metribuzin is relatively soluble in water (1.2 g/L at 20°C), and is not strongly bound to most soils (Bowman, 1991). Nichols et al. (1982) and Peter and Weber (1985) reported that metribuzin was more mobile in light textured soils than was atrazine (atrazine is the most commonly used pesticide in Wisconsin and atrazine contamination of groundwater is common throughout the state). Bowman (1991) reported that metribuzin and its degradates are more mobile than atrazine in Plainfield sand soils (based on field lysimeter studies). Plainfield sand soils are similar to soils found in the central sands region of Wisconsin where groundwater samples were obtained for the present study.

Very little is known about metribuzin metabolites in groundwater. Most work on metribuzin degradation has been done on soils and plants. Apparently, degradation into metabolites in soils is primarily microbially mediated

(Sharom and Stephenson, 1976; Moorman and Harper, 1989; Bowman, 1991). The U.S. Environmental Protection Agency (1992) report a soil half life of 40 days, a value similar to that reported by others (Kempson-Jones and Hance, 1979; Savage, 1977). Obviously, microbial degradation rates will vary among soils and with soil temperature and moisture. There appears to be no information on the degradation rates of metribuzin in groundwater, either microbial or otherwise. If microbial degradation is the key mechanism for degradation, it is likely that most metabolite formation occurs in the surface soils.

Of the metribuzin metabolites, Bowman (1991) reported that DADK was the most mobile based on lysimeter tests with supplemental watering. DK was more mobile than DA, which in Plainfield sand had similar mobilities to the parent metribuzin. Bowman (1991) also reported that, based on his experiments, the primary degradation pathway was through the DK intermediate rather than the DA intermediate.

Monitoring of metribuzin and metabolites in water has been sparse. In an early work Muir and Baker (1976) reported the presence of metribuzin in tile-drain water. Brown et al. (1984) measured metribuzin in runoff water from wheat fields. Recently, Thurman et al. (1990) reported metribuzin in surface water. Some state agencies have done some monitoring for metribuzin (e.g., Postle, 1991) and the U.S. Environmental Protection Agency has recently initiated some groundwater herbicide monitoring programs that include metribuzin (U.S. Environmental Protection Agency, 1990 and 1992). However, overall, little information was found in the literature on metribuzin in water.

The purpose of the present study was to measure metribuzin, and in particular metabolites of metribuzin, in Wisconsin groundwater susceptible to metribuzin contamination. In making metabolite measurements, improvements in methods of analysis were sought. Results of analytical method development, as well as the analysis of several wells suspected of containing metribuzin, are

reported here. This study was part of a larger effort to measure pesticide metabolites in Wisconsin groundwater.

Method Development

Although several investigators have reported methods to measure metribuzin metabolites (Thornton and Stanley, 1977; Parker et al., 1983; Brown et al., 1984; Frear et al., 1985; Hatzios and Penner, 1988; Parker et al., 1988; Pereira et al., 1990), poor recoveries were obtained (especially for DK and DADK). Consequently, a variety of techniques were evaluated. The technique described below, utilizing solid phase extraction (SPE) and high performance liquid chromatography (HPLC), was found to provide the best overall results. In conducting the analyses, all solvents (including water), and glacial acetic acid, were of HPLC grade.

The first step of the technique was to acidify 1 L of sample with 3.00 g of glacial acetic acid (acidification greatly improved recovery of DA). The sample was then thoroughly mixed and filtered through a 0.45 μm Millipore filter. Sodium chloride (ACS grade; muffled at 450°C for four hours) was then dissolved in the acidified sample (150 g of NaCl per L).

Next, SPE was used to extract the pesticides from the sample and subsequently concentrate them. A Supelco vacuum manifold SPE unit, allowing multiple simultaneous extractions, was used for these extractions. The manifold was fitted with Supelco ENVI-18 (1 g) SPE cartridges. All cartridges were conditioned prior to analysis with methanol followed by HPLC grade water.

Details of the conditioning process are as follows. Initially, 4.0 mL of methanol were added to cartridges connected (with ports closed) to the manifold. The manifold ports were then opened slightly until methanol had completely saturated the bed of solid phase material. The ports were then closed, and the methanol was allowed to stay in contact with the cartridge bed for ten minutes. At this point, the ports were opened and the methanol was slowly drained from each cartridge under gravity or a minimal vacuum. Flow was stopped as the methanol level approached the top of the bed; then, 4.0 mL

aliquots of HPLC grade water were added to each cartridge. The water conditioning procedure was the same as for methanol.

Following conditioning, the cartridges were filled with water and connected in serial pairs. Each pair was connected to the manifold. A Teflon™ tube was run from the top of each cartridge pair down to the bottom of the bottles of prepared sample. Sample water was slowly drawn through the tubing (for about 200 minutes) under a manifold vacuum maintained steadily at just under 130 mm Hg. To minimize variability the cartridge bed was kept covered with sample and the manifold pressure was held constant.

Once the above step was completed, the manifold vacuum was increased to 380 to 500 mm Hg and held there for one hour to dry the samples. The sample cartridges were then eluted with 4.0 mL methanol, which was added in two 2 mL aliquots. The first aliquot was allowed to thoroughly soak the cartridge material for ten minutes before being drawn into collection tubes under a slight vacuum. The second aliquot of methanol was run through under increased vacuum (50 mm of Hg under an air stream to about 0.5 mL and adjusted to 1.0 mL with HPLC grade water in 1.00 mL volumetric tubes.

Analyses of the extracts were done by HPLC (Hewlett Packard Model 1090 with a continuous wavelength diode array detector). Compounds were separated using a 25 cm x 4.6 mm Phase Sep reversed phase Spherisorb C18 bonded phase column and absorbance was measured at 254 nm. Mobile phase solvents were degassed by helium sparging and were vacuum filtered through a 0.45 μm filter. The eluent composition was a 50:50 methanol and acidified water (3.00 g acetic acid/L of water) mixture. The flow rate was 1.0 mL/min, and the column was kept at 42°C. Integration and plotting of chromatograms was done by a Hewlett Packard 3390A integrator.

Confirmations of all detects were made by reinjecting samples through a different column (J&W Scientific cyano bonded reversed column) of the same dimensions. HPLC parameters were the same except that eluant flow was 0.65 mL/min. Qualitative confirmations were achieved by comparing retention times with those of external standards. The limit of detection (LOD) for each of

the analytes was determined from replicates, each spiked so that the final concentration was three to five times the LOD (Longbottom and Lichtenberg, 1982). Analyses were always conducted at least in duplicate. Percent recoveries were also determined by adding a known amount of standard to water (Madison tap water or HPLC grade distilled water) and carrying this sample through the whole procedure.

Sample Collection

Samples were collected from Wisconsin Department of Natural Resources monitoring wells using standard sampling procedures (Wisconsin Department of Natural Resources, 1989). Samples were collected in 1 L amber glass bottles capped with Teflon™-lined screw caps. Most of the groundwater sites were known to have metribuzin contamination. Replicate samples were drawn from each well and field blanks were prepared at each site with laboratory tap water. Samples were iced down in the field and were kept refrigerated at 4°C prior to analysis. Most samples were analyzed within two weeks of collection. Brown et al. (1984) have reported metribuzin and DADK to be relatively stable over long periods.

Results

The results of the analysis of the groundwater samples are presented in Table 1. Of the 20 wells tested, 14 contained the parent metribuzin compound. However, the deaminated metabolite, DA, was found in 18 of the samples. The DK and DADK metabolites were found in 13 and 16 of the samples, respectively.

The parent metribuzin concentration ranged to 10.2 μg/L. The highest concentration of the metabolites was about an order of magnitude less. Maximum concentrations of DA, DK, and DADK were 1.56, 0.54 and 1.88 μg/L, respectively.

The mean recoveries in Madison tap water and HPLC grade distilled water are given in Table 2. From the table it can be seen that recoveries averaged about 111% for metribuzin, 93% for DA, 35% for DK and 63% for DADK. Precision was generally good, as duplicate analyses were nearly always within 5% of each other.

Table 1. Metribuzin and metabolites of metribuzin measured in twenty Wisconsin wells

	µg/L			
Well #	Metribuzin	DA	DK	DADK
1	0.0	0.4	0.0	0.0
2	0.3	1.2	0.0	0.5
3	0.0	1.1	0.2	0.5
4	0.0	0.0	0.0	0.0
5	0.7	0.9	0.1	0.4
6	0.2	0.9	0.2	0.3
7	0.4	1.3	0.0	0.6
8	0.8	1.1	0.2	1.0
9	0.4	0.9	0.2	0.6
10	0.5	1.3	0.3	1.1
11	0.0	1.0	0.1	0.5
12	2.7	1.6	0.3	1.9
13	2.5	0.8	0.2	0.5
14	0.0	0.8	0.0	0.4
15	3.2	0.1	0.2	0.0
16	8.9	0.2	0.3	0.6
17	10.2	0.7	0.5	0.6
18	0.5	0.4	0.1	0.6
19	0.4	0.4	0.0	0.6
20	0.0	0.0	0.0	0.0

Table 2. Mean Percent Recovery and Standard Deviation (SD) of Metribuzin and Metabolites Spiked in Madison Tap Water and HPLC Grade Distilled Water

	% Recovery ± SD	
	Tap Water	HPLC Grade Distilled Water
Metribuzin	109.6 ± 11.3	11.6 ± 11.0
DA	92.4 ± 4.7	93.5 ± 4.1
DK	34.5 ± 1.7	34.8 ± 1.7
DADK	62.2 ± 4.3	64.1 ± 1.3

The limit of detections (LODs) determined for the analytes were 0.19, 0.24, 0.14 and 0.28 μg/L for metribuzin, DA, DK and DADK, respectively. Thus, the LODs determined were on the order of a few tenths of a μg/L. When evaluating the LOD for DK, and to some extent DADK, the low recoveries should be noted.

Discussion

The SPE/HPLC method described here was found to be useful for measuring metribuzin compounds in groundwater. The method was precise and recoveries were high for metribuzin and DA, and generally higher than reported in the literature for DADK and DK. Despite efforts to improve the recover of DK, it was such (on the order of 35%) that it can only be considered a qualitative or semi-quantitative test. Recoveries from samples spiked in Madison tap water and HPLC grade distilled water were essentially the same (Table 2). Madison tap water is pumped from several high capacity wells and is quite hard (~300 mg/L as $CaCO_3$). Similar recoveries from tap water and distilled water, as well as from recovery analyses performed on groundwater collected from the Central Sands and Wisconsin Valley regions, suggests the absence of groundwater interferences.

Recoveries were improved by adding 150 g of NaCl to the sample prior to extraction (smaller amounts of NaCl led to poorer recoveries). Shevchuk et

al. (1987) also reported improved extractions using a salting-out procedure. The use of two 1 g C18 cartridges in series also improved the extraction efficiency (relative to using only a single cartridge). Another important variable was the vacuum pressure. When the pressure was carefully controlled, the precision of the test was improved.

Very little information was found in the literature on the limit of detection for metribuzin and metabolites. The limit of detection determined here is substantially lower than reported by the U.S. Environmental Protection Agency (1990) in their national survey of pesticides in drinking water wells (Table 2). Di Corcia and Marchetti (1991) reported a lower detection limit for metribuzin using an HPLC technique, but it was unclear how they determined their detection limit (it may represent an instrument detection limit).

Metribuzin and metribuzin metabolites were found in all but 2 of the 20 wells sampled. The parent herbicide was found in 14 of the wells, while DA, DK and DADK were found in 18, 13 and 16 of the wells, respectively. Thus, metabolites were found in four wells even though the parent compound was absent.

Very little data exist on metribuzin in natural waters to allow comparison to the results of this study. Muir and Baker (1976) reported metribuzin concentrations ranging from not detected to 1.65 μg/L in tile drain water. Cohen et al. (1986) reported three observation wells in northern Iowa had metribuzin concentrations ranging from 0.09 to 4.35 μg/L. Postle (1991) reported metribuzin concentrations as high as 53.5 μg/L in Wisconsin wells, similar (from the same area) to the wells studied here. The 53.5 μg/L concentration was about twice as high as the next highest value. Note from Table 1 that the highest concentration for metribuzin in the present study was 10.2 μg/L. No data on metribuzin metabolites in natural water samples was found to compare to the results of the present study.

It is interesting to note the distribution between the parent herbicide and metabolites for the waters analyzed. Figure 2 shows total metribuzin plus the relative distribution of the parent and metabolites. All metabolite

concentrations are expressed as μg/L of metribuzin (i.e., metabolite concentrations are expressed in terms of a common constituent to allow a total concentration to be determined). From the figure it appears that the ratio of parent to metabolites is higher when the total concentration is high. When total concentration are low (less than about 2 μg/L), the metabolites comprise most or even all of the total metribuzin concentration. Such a pattern suggests that those wells with high parent concentrations may have been influenced by recent field applications of metribuzin, in that little degradation of the parent herbicide has occurred. It would be of interest (although beyond the scope of the present study) to try to correlate metribuzin and metabolite ratios with dates of field application of the herbicide.

In conducting the analysis of groundwater, samples were passed through a 0.45 μm filter to remove particulates. In Wisconsin, at least for samples collected by the Wisconsin Department of Natural Resources, it is common practice to filter groundwater samples prior to any type of analysis. The reasoning is that a filtered sample is more representative of groundwater, the presence of particulates often being a sampling artifact. In the present study filtration also is advantageous in that it removes particulates that might otherwise clog or retard flow through the SPE cartridges. Whether any metribuzin compounds might be lost with the particles is not known, but it appears unlikely since, as discussed previously, metribuzin does not strongly bind to particles. Further, acidification prior to filtration should have promoted solubilization of the metribuzin compounds. More research should be directed in this area.

The current study is one of the few to report metribuzin, and particularly metribuzin metabolites, in groundwater. Documentation of the existence of these metribuzin metabolites is important, since, at least in Wisconsin, total pesticides (parent plus metabolites) are considered in setting drinking water standards. For example, the Wisconsin standard for atrazine in groundwater is 3.5 μg/L (Sec. NR 140.10, Wisconsin Administrative

Code). However, Wisconsin's groundwater law allows for standards to be established "in its original form, or as a metabolite or a degradation product" [Sec. 160.02(8), Wisconsin Statutes]. Thus, agencies establishing and enforcing these standards are now considering the presence of breakdown products in their assessment of the standard (Wisconsin Department of Agriculture, Trade and Consumer Protection, 1991). They are assuming metabolites, such as the dealkylated atrazine compounds, to be as toxic as the parent compound.

Currently, the Wisconsin enforcement standard for metribuzin is 250 μg/L, while the preventive action limit (not an enforcement standard, but rather a warning that the contamination should be monitored or action taken to prevent further contamination). Thus, the concentrations of metribuzin found in this study are below the current enforcement standard and the preventive action limit, even if total metribuzin is considered. However, the atrazine standard in Wisconsin is an order of magnitude lower than the metribuzin standard, and effectively lower than that considering atrazine metabolites. Further, toxicity data on metribuzin is limited, and virtually no data exist for metribuzin metabolite toxicity. It would not be surprising to the authors if standards for metribuzin in drinking water are lowered in the future and metribuzin violations occur. Even with the current standard, metribuzin and metabolites can contribute to the total pesticide burden, given that groundwaters are often contaminated by a variety of residues.

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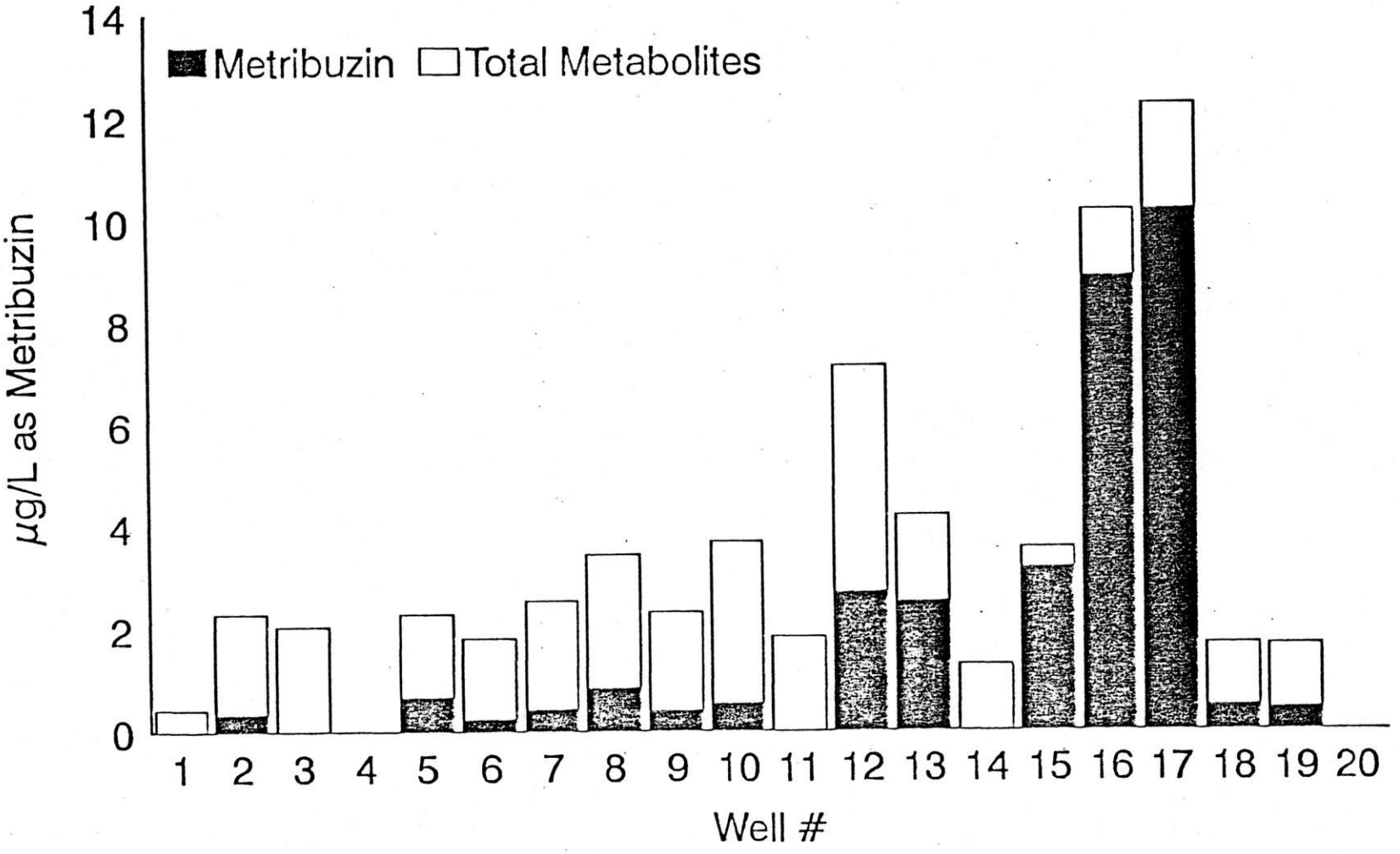
Metribuzin
Total Metabolites
µg/L as Metribuzin
0
2
4
6
8
10
12
14
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Well #