Bull. Environ. Contam. Toxicol. (1992) 49:1–9


Environmental Contamination and Toxicology

# Atrazine, Alachlor, and Carbofuran Contamination of Well Water in Central Maine

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Ever since 1979 when aldicarb was found in well water in Long Island (Zaki et al. 1982), there has been an increased effort to determine the extent of pesticide contamination of groundwater (Graham, 1991; Anonymous, 1991). Three pesticides - alachlor, atrazine and carbofuran - have been shown to be present in well water from several areas in the United States (Anonymous, 1991; Thurman et al. 1990; Feng et al. 1990; Anonymous, 1985; Chesters et al. 1989).

Because of groundwater contamination and possible health effects, these 3 pesticides have had Maximum Contaminant Level Goals (MCLG) proposed by the EPA for primary drinking water sources. They are zero for alachlor, 3 ppb for atrazine, and 40 ppb for carbofuran (U.S. EPA, 1989). In order to meet these proposals states are looking towards rapid monitoring capabilities at a minimal cost. One such technique may be enzyme immunoassay (EIA). Recently it has been used with great success in monitoring pesticides in groundwater (Bushway et al. 1988; Thurman et al. 1990; Feng et al. 1990; Kinderwater et al. 1990).

This study was performed to 1) determine the extent of atrazine, alachlor and carbofuran contamination in private well water in central Maine and 2) evaluate the utility of immunoassay techniques as a part of a groundwater monitoring/compliance program for state management plans.

## MATERIALS AND METHODS

During 1990 fifty-eight private wells (all primary sources of drinking water) in the central Maine area were sampled 3 times. Sampling periods were 1) late May through early June, 2) early August, and 3) late October through early November. Five towns made up the sampling area - East Corinth, Charleston, Exeter, Corrina and Cambridge. Ten of these wells were on farms (#14, 15, 19, 23, 25, 26, 33, 51, 62 and 63) while the other 48 were from nonfarm

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houses. All but 5 of the wells were drilled (#4, 5, 24, 27 and 46); these were hand dug. Water was sampled at the tap unless a water purifying system was present, in which case the sample was taken before the purifier. The water was allowed to run for 5-10 minutes before two solvent cleaned canning jars were filled for HPLC analysis. For immunoassay analysis two 20 ml scintillation vials were filled. Samples were always kept cool or frozen until analysis. The immunoassay samples were analyzed immediately while the HPLC samples were concentrated on C18 Sep-Paks within 2 days of storage. Eluent from the Sep-Paks were stored frozen until analyzed.

For each of the three sampling periods, nine well water samples (the well waters were previously determined to be free of the 3 pesticides at 0.04 ppb) were spiked at 0.5 ppb, 1.0 ppb and 5.0 to 10 ppb, 3 at each concentration. These samples were used as controls since they were treated like the actual well samples as far as transportation, handling and analysis.

Pesticides were obtained from the EPA (Research Triangle Park, NC). All solvents were HPLC grade (Fisher Scientific, Pittsburgh, PA).

Analysis of alachlor, atrazine and carbofuran was performed by immunoassay using EnviroGard™ EIA kits (Millipore Corp., Bedford, MA). Protocol for each kit was followed according to the manufacturers instructions with two exceptions. First, repeater pipette systems (Gilson Inc., Middleton, WI) were used to deliver solutions to the tubes instead of the dropper bottles, and second, 8 tubes were run simultaneously with optical densities at 450 nm being determined by transferring 200 $\mu$l aliquots to micro-titer wells. Standards for all three pesticides were prepared and assayed according to the method of Bushway et al. "in press". A set of standards were run at the beginning and end of each day of the assay with the averages used to prepare the standard curve.

To verify the immunoassay results, a solid phase extraction method developed by Brooks et al. 1989 was employed with two modifications. First, the two ml elution from the C18 cartridge was evaporated to dryness under nitrogen. The remaining residue was dissolved in 0.4 ml of high-performance liquid chromatography (HPLC) mobile phase and filtered through a 0.2 $\mu$ nylon syringe filter (Gelman Inc., Ann Arbor, MI). Second, instead of using gas chromatography for quantification, HPLC was employed.

HPLC was performed using a Valco injector (Vicci Instruments, Houston, TX) with a 25-$\mu$l loop, a Waters 510 pump (Milford, MA) and a Hewlett-Packard 1040A photodiode array detector (Avondale, PA). The column was either an Ultracarb 530 ODS, 150 mm x 4.6 mm (Phenomenex, Inc., Torrance, CA) or an Asahipak ODP-50, 150 mm x 6.0 mm (Advanced Separations Technologies, Inc., Whippang, NJ). Mobile phase was acetonitrile/water/monoethanolamine (420 ml + 180 ml + 0.05 ml v/v) with a flowrate of 1 ml/min. Detection was at 205 nm for carbofuran and alachlor and 224 nm for atrazine. Whenever

possible, ultraviolet spectral scans were run on the pesticide chromatographic peaks to check the purity and confirm their identity.

## RESULTS AND DISCUSSION

Of the 67 well samples analyzed three times during 1990, 9 were spiked and 58 were nonspiked. The nonspiked samples came from 5 towns in central Maine encompassing 3 of the 16 counties of Maine. The exact region sampled is shown in Figure 1. Agriculture in this area is comprised mainly of dairy and potato farms. Only 5 wells were hand dug wells and all wells were private drinking wells, mostly on nonfarm land (82.8% of the wells), but with farms upslope from the wells sampled.

The results of the pesticide analysis of the wells are given in Tables 1-3. Triazine results are shown in Table 1 and are given as the amount of atrazine found since the HPLC method was specific for atrazine. Eighteen wells (31%) of the nonspiked wells demonstrated triazine contamination at least once during the 3 sample periods. Eleven of these 18 wells were found to be contaminated during all periods sampled while six were found to have triazines at one sampling period and 1 well was contaminated during 2 sampling periods. Two samples, which were from different wells, contained more atrazine than the MCGL of 3.0 ppb. One of these wells was located on a potato farm while the other was a nonfarm well. Most triazine positive wells had less than 0.60 ppb triazines. In three cases, samples were found to be triazine positive by EIA, but atrazine negative by HPLC. However, all three samples (#23-1, #23-2, #15-3) contained unknown compounds that had UV spectra typical of triazine pesticides with UV maxima at 224 nm and 254 nm. Most likely those compounds were other triazines and/or metabolites of atrazine, both of which have cross-reactivity to the atrazine antibody (Bushway et al. 1988).

As discussed above, most samples that were triazine positive contained atrazine. Therefore, a linear regression was done on the atrazine positive samples to compare both techniques. The equation obtained for 60 samples including the spiked well samples was $y = 1.04x + 0.18$ with a correlation coefficient of 0.97. The correlation between HPLC and EIA for atrazine concentration in well water was excellent making this a good screening technique that is rapid and inexpensive. This correlation agrees with the results obtained by Thurman et al. 1990 in their study of groundwater from the Midwest. The other non-spiked wells (40) or 69% of the total showed no detectable levels of triazines or atrazine for all 3 sample periods by both techniques. Again this reflects how effective the triazine immunoassay was, since nearly 70% of the samples were negative; had testing been performed by a conventional chromatographic technique in a commercial laboratory, these "negative" results would have cost at least $2,000.

Carbofuran results are given in Table 2. Only 4 nonspiked wells were shown

Table 1. Atrazine concentration in 67 nonspiked and spiked private wells from central Maine, sampled 3 times in 1990.

| Well Number | Atrazine Found (ng/ml) Sample Set 1 | | Sample Set 2 | | Sample Set 3 | |
|---|---|---|---|---|---|---|
| 1 | 0.13[1] | (0.11)[2] | 0.25 | (0.09) | 0.23 | (0.10) |
| 2* | 9.8 | (8.4) | 10 | (7.5) | 8.5 | (9.3) |
| 4 | 0.53 | (0.40) | 0.50 | (0.10) | 0.59 | (0.28) |
| 5 | 0.20 | (0.16) | 0.37 | (0.06) | NS | (NS) |
| 7* | 0.37 | (0.62) | 0.50 | (-) | 0.40 | (0.34) |
| 10 | ND | (ND) | 0.10 | (-) | ND | (ND) |
| 12 | ND | (ND) | 0.10 | (-) | ND | (ND) |
| 15 | ND | (ND) | ND | (ND) | 0.65 | (ND) |
| 17 | 0.11 | (0.08) | ND | (ND) | ND | (ND) |
| 19 | 0.25 | (0.45) | 0.32 | (0.14) | 0.44 | (0.40) |
| 20 | 0.95 | (-) | ND | (-) | ND | (ND) |
| 22* | 9.8 | (10) | 10 | (10) | 5.0 | (6.8) |
| 23 | 0.90 | (ND) | 0.83 | (ND) | ND | (NS) |
| 26 | 3.5 | (3.2) | 0.27 | (0.19) | 0.29 | (0.18) |
| 29* | 1.3 | (1.1) | 1.0 | (0.82) | 0.90 | (0.68) |
| 31* | 0.50 | (0.62) | 0.58 | (0.42) | 0.42 | (0.37) |
| 40* | 0.60 | (0.53) | 0.41 | (0.50) | 0.40 | (0.34) |
| 45* | 7.5 | (8.7) | 10 | (7.6) | 10 | (6.5) |
| 50* | 1.2 | (0.85) | 0.93 | (0.72) | 0.98 | (0.64) |
| 51 | 0.16 | (0.06) | 0.55 | (0.40) | 0.26 | (0.11) |
| 52 | 0.25 | (0.27) | 0.25 | (0.14) | 0.28 | (0.14) |
| 54 | 0.78 | (0.75) | 5.0 | (4.1) | 1.2 | (0.78) |
| 55 | 1.0 | (0.76) | 1.1 | (0.80) | 0.40 | (0.36) |
| 57* | 0.95 | (0.63) | 0.93 | (0.80) | 1.1 | (0.58) |
| 63 | ND | (ND) | ND | (ND) | 0.10 | (0.14) |
| 64 | 0.15 | (0.10) | 0.10 | (0.04) | ND | (ND) |
| 66 | 0.15 | (0.15) | 0.37 | (-) | 0.26 | (-) |

1 = immunoassay value
2 = HPLC value
* = sample was spiked well water (well water #7, 31 & 40 were spiked at 0.5 ppb; well waters #29, 50 & 57 were spiked at 1.0 ppb; well waters #2, 22 & 45 were spiked at 5 to 10 ppb

ND = none detected at a detection limit of 0.10 ppb for immunoassay and 0.04 ppb for HPLC; NS = not sampled; (-) = not analyzed

All other private wells sampled (numbers 1 through 67) not listed above showed no detectable levels of atrazine by both immunoassay and HPLC.

Table 2. Carbofuran concentration in 67 nonspiked and spiked private wells from central Maine, sampled 3 times in 1990.

| Well Number | Carbofuran Found (ng/ml) Sample Set 1 | | Sample Set 2 | | Sample Set 3 | |
|---|---|---|---|---|---|---|
| 2* | 10[1] | (7.6)[2] | 7.9 | (6.3) | 10 | (10) |
| 7* | 0.56 | (0.36) | 0.36 | (-) | 0.56 | (0.50) |
| 22* | 10 | (4.8) | 10 | (6.1) | 5.8 | (6.9) |
| 24 | 0.25 | (0.08) | ND | (ND) | NS | (NS) |
| 29* | 1.1 | (0.43) | 1.1 | (1.1) | 1.2 | (0.81) |
| 31* | 0.54 | (0.39) | 0.76 | (0.42) | 0.52 | (0.52) |
| 40* | 1.0 | (0.38) | 0.61 | (0.48) | 0.62 | (0.38) |
| 42 | 0.25 | (ND) | ND | (ND) | NS | (NS) |
| 45* | 10 | (-) | 9.6 | (5.4) | 10 | (6.6) |
| 50* | 1.1 | (-) | 1.2 | (1.3) | 0.90 | (0.87) |
| 57* | 1.2 | (-) | 1.1 | (1.5) | 0.72 | (0.55) |
| 62 | ND | (ND) | ND | (ND) | 0.20 | (0.14) |
| 63 | 1.2 | (0.38) | ND | (ND) | ND | (ND) |

1 = immunoassay value
2 = HPLC value
* = sample was spiked well water (well water #7, 31 & 40 were spiked at 0.5 ppb; well waters #29, 50 & 57 were spiked at 1.0 ppb; well waters #2, 22 & 45 were spiked at 5 to 10 ppb
ND = none detected at a detection limit of 0.10 ppb for immunoassay and 0.04 ppb for HPLC; NS = not sampled; (-) = not analyzed
All other private wells sampled (numbers 1 through 67) not listed above showed no detectable levels of carbofuran by both immunoassay and HPLC.

to be carbofuran positive (6.9%) by immunoassay and in just one of the three sampling periods. Three of the 4 were also shown to be carbofuran positive by HPLC. The other sample which was positive just by immunoassay could have had either 3-OH carbofuran or 3-keto carbofuran present since both show cross-reactivity toward the carbofuran antibody, but would not have been detected by the HPLC method. None of the contaminated wells approached the MCGL of 40 ppb for carbofuran.

A linear regression was performed on the positive samples including the spiked samples in order to compare both methods. The regression equation was $y = 1.25x + 0.26$ with a correlation coefficient of 0.94. A slope of 1.25 indicates that the immunoassay results are slightly higher than HPLC. All other nonspiked well samples (92.1% of the wells) contained no detectable carbofuran by both procedures. Like the triazine samples there were no false negatives, thus making the carbofuran an excellent screening method.

Table 3. Alachlor concentration in 67 nonspiked and spiked private wells from central Maine, sampled 3 times in 1990.

| Well Number | Alachlor Found (ng/ml) Sample Set 1 | | Sample Set 2 | | Sample Set 3 | |
|---|---|---|---|---|---|---|
| 2* | 10[1] | (7.2)[2] | 7.8 | (5.5) | 8.0 | (-) |
| 7* | 0.74 | (0.59) | 0.34 | (-) | 0.56 | (0.37) |
| 17 | 0.25 | (0.10) | 0.15 | (ND) | 0.13 | (ND) |
| 19 | 0.20 | (0.05) | 0.15 | (ND) | 0.17 | (ND) |
| 22* | 10 | (4.7) | 5.0 | (4.5) | 6.3 | (5.8) |
| 29* | 1.9 | (0.96) | 0.75 | (0.65) | 0.93 | (0.70) |
| 31* | 0.78 | (0.68) | 0.37 | (0.41) | 0.56 | (0.40) |
| 32 | ND | (ND) | ND | (ND) | 0.11 | (ND) |
| 40* | 0.64 | (0.33) | 0.51 | (0.51) | 0.65 | (0.31) |
| 45* | 10 | (-) | 10 | (4.8) | 9.4 | (5.8) |
| 50* | 1.1 | (0.71) | 1.7 | (0.78) | 1.1 | (0.54) |
| 51 | 0.14 | (-) | 0.25 | (ND) | ND | (ND) |
| 52 | 0.14 | (0.05) | 0.36 | (ND) | 0.16 | (ND) |
| 54 | 0.10 | (-) | 0.31 | (0.14) | 0.11 | (ND) |
| 55 | 0.18 | (0.31) | 0.20 | (0.21) | 0.25 | (ND) |
| 57* | 1.1 | (0.75) | 0.95 | (0.93) | 1.2 | (0.47) |
| 60 | ND | (ND) | ND | (0.13) | ND | (ND) |

1 = immunoassay value
2 = HPLC value
* = sample was spiked well water (well water #7, 31 & 40 were spiked at 0.5 ppb; well waters #29, 50 & 57 were spiked at 1.0 ppb; well waters #2, 22 & 45 were spiked at 5 to 10 ppb
ND = none detected at a detection limit of 0.10 ppb for immunoassay and 0.04 ppb for HPLC; NS = not sampled; (-) = not analyzed
All other private wells sampled (numbers 1 through 67) not listed above showed no detectable levels of alachlor by both immunoassay and HPLC.

Results from the alachlor analysis are shown in Table 3. Seven nonspiked wells (12.1%) were found to contain alachlor by EIA. Of these seven wells, 5 were found to be alachlor positive all three sample periods while 1 was positive twice and the other once. Unlike the triazine and carbofuran assays, results of the alachlor study contain many samples that are positive by immunoassay, but not by HPLC. In fact, of the 18 positive well samples by immunoassay, just six were found to have alachlor by HPLC. This discrepancy could be caused by metabolites of alachlor, but we were unable to obtain metabolites to test for their cross-reactivity. Also, one well water was alachlor positive by HPLC but not by immunoassay. Since the MCGL for alachlor is 0, therefore 19 well samples (13.8%) of the nonspiked wells contained more alachlor than the

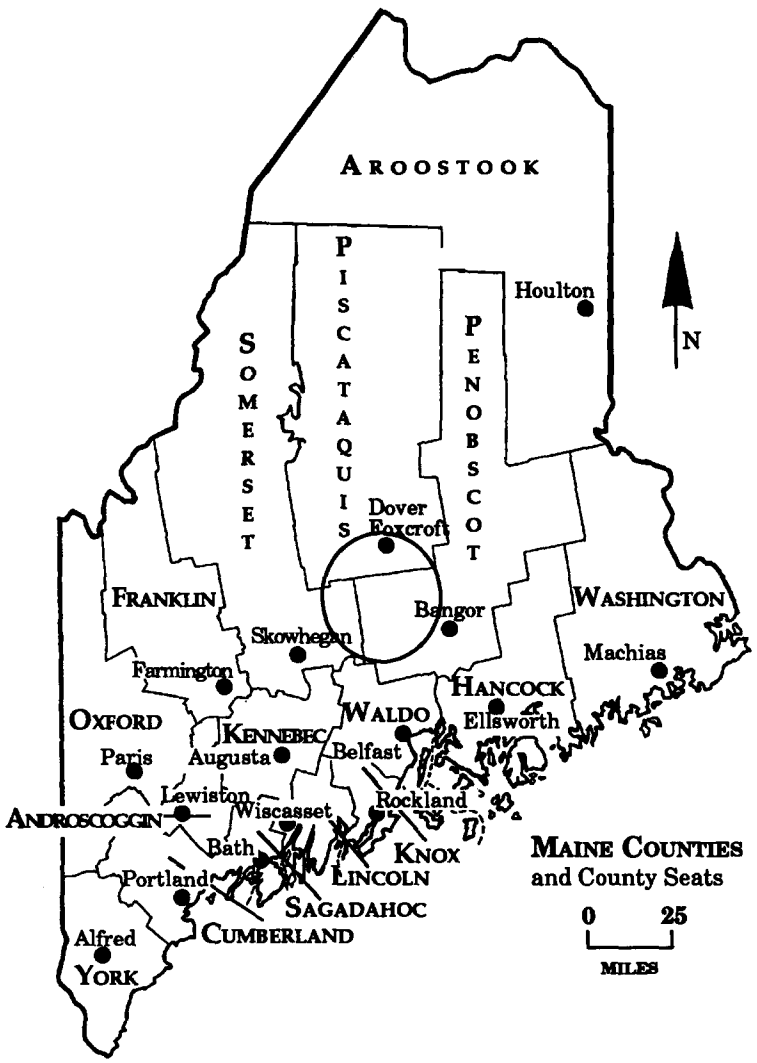


Figure 1. Map depicting sampling sites for well water study.

MCGL. All other nonspiked wells were shown to have no detectable alachlor present at anytime during sampling by either immunoassay or HPLC.

The correlation coefficient from the comparison of immunoassay and HPLC for the 40 positive alachlor samples was 0.96 and the regression equation was y = 1.49x 0.083. The slope indicates a high bias for the immunoassay values. However, the results indicate that the alachlor immunoassay would be sufficient for a screening test even though there appear to be many false positives. More work needs to be done with the immunoassay using alachlor groundwater metabolites in order to determine whether in fact EIA was providing true false

positives or was actually detecting the presence of real alachlor breakdown products. This correlation was better than that obtained by Feng et al. 1990 for their Midwest water study, but the level of false positive rate was about the same.

There were 7 nonspiked wells in this study that were shown to have multiple pesticide contamination (Tables 1, 2 and 3). Well numbers 17, 19, 51, 52, 54 and 55 were found to have alachlor and atrazine present, while well number 63 was shown to contain carbofuran and atrazine. These wells were located in the towns of East Corinth, Corinna and Cambridge, which were the areas of the worst contamination from these 3 pesticides. Two of these wells (#19 and 51) were located on farms while the other 4 were not.

Our results provide evidence that these EIA's are a simple, suitable, cost-effective screening method for the presence of atrazine, carbofuran and alachlor in groundwater. The best results were for atrazine and carbofuran, but alachlor was adequate although there were apparently a few false positives and 1 false negative. However, the false positives should be tested for possible metabolites that may cross-react with the antibody. The speed and low cost of EIA is difficult to match. Although the number of contaminated wells seems low, only a small area of the State was sampled, thus the results represent sufficient contamination so that a wider area should be sampled using EIA as a screen.

Acknowledgments. We thank the Maine Board of Pesticides Control for their support. This paper is #1611 of the Maine Agricultural Experiment Station.

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Received August 10, 1991; accepted January 13, 1992.