(32) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic: London, 1985.
(33) Boughey, M. T.; Duckworth, R. M.; Lips, A.; Smith, A. L. *J. Chem. Soc., Faraday Trans. 1* **1978**, *74*, 2200.
(34) Hough, D. B.; White, L. R. *Adv. Colloid Interface Sci.* **1980**, *14*, 3.
(35) Onarova, K. I.; Musabekov, K. B.; Izimov, A. I.; Skachkova, A. L.; Kenzhebaeva, G. K. *Colloid J.* **1986**, *48*, 176.
(36) Vincent, B. *Spec. Discuss. Faraday Soc.* **1970**, *1*, 78.

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Comparison of an Enzyme-Linked Immunosorbent Assay and a Gas Chromatographic Procedure for the Determination of Molinate Residues

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An enzyme-linked immunosorbent assay (ELISA) was compared to a gas chromatographic method for the analysis of the thiocarbamate herbicide molinate (*S*-ethyl hexahydroazepine-1-carbothioate). Apparent recoveries from water spiked at 1 ppb to 1 ppm levels were comparable when liquid–liquid extraction was used. Solid-phase extraction was also examined and apparent recoveries by both ELISA and gas chromatography (GC) were comparable to each other as well as to the liquid–liquid extraction method. Methanol, acetonitrile, and ethyl acetate were equally effective in eluting molinate from solid-phase columns. An excellent correlation was obtained between the ELISA and GC method for field-treated water samples extracted by using the solid phase method and either ethyl acetate or methanol as the eluting solvent. Air and soil samples from this same study correlated well when analyzed by ELISA or GC, but ELISA results for soil were generally higher than GC data and of slightly lower precision than GC. Tests with a coated plate, pipettors, and the plate reader amounted to 8.0% error, the majority of which was attributable to the coating antigen binding and to antigen–antibody reactions.

INTRODUCTION

The enzyme-linked immunosorbent assay (ELISA) has been shown to be a useful residue analysis method (*1–8*). It is most suited to analysis of molecules that are difficult to analyze by conventional methods, such as the herbicide paraquat (*7*) and the benzoylphenylurea insecticide diflubenzuron (*9*), and to higher molecular weight, water-soluble compounds. The herbicide molinate (*S*-ethyl hexahydroazepine-1-carbothioate), however, is a low molecular weight, relatively volatile, and hydrophobic compound, and it contains a relatively labile thiocarbamate bond. The conventional analysis method (by gas–liquid chromatography, GC) is sensitive and well characterized (*10, 11*). Molinate thus provided a challenge as a target for immunoassay development. An ELISA was developed in this laboratory (*12*) in order to improve sample throughput and to conduct measurements in the field. These are important goals because molinate is used extensively in rice culture in ways which may, if not monitored carefully, contaminate water released from the rice fields and air in the vicinity of the rice fields (*13*).

In this study we examined the ELISA method for molinate for sources of error and verified its accuracy and precision against a reference GC method. We compared extraction efficiency, apparent recovery, matrix effects, other sources of error (such as pipettors) and performance using field samples of water, soil, and air. Since this procedure was designed for monitoring water from rice fields, drainage basins, and other surface waters where relatively low residues might be encountered, a solid-phase extraction–concentration method was also examined and compared to the commonly used wet extraction method. Since molinate can be eluted from these columns with the water-miscible solvents acetonitrile or methanol, this method is very adaptable to use with ELISA.

EXPERIMENTAL SECTION

Reagents. The immunizing antigen was prepared by conjugating the mercaptopropionic acid derivative of molinate to hemocyanin by the mixed anhydride method, and the anti-molinate antibodies were raised in New Zealand white rabbits as described by Gee et al. (*12*). Goat anti-rabbit IgG conjugated to alkaline phosphatase was purchased from Miles Scientific. The *p*-nitrophenyl phosphate enzyme substrate, 96 well microtiter plates, and C_{18} cartridges were purchased from Sigma Chemical Co., Flow Laboratories, and Analytichem International, Inc., respectively. Molinate (96.4%) and [^{14}C]molinate (10.4 μCi/μmol) were generously provided by ICI (formerly Stauffer Chemical Co.).

Extraction of Water. For liquid–liquid extraction, 500 mL of tap water fortified with 10 ppb, or 100 mL fortified with 100 ppb or 1.0 ppm, was extracted by shaking 3 times with methylene chloride (2:1 (v/v)) then once with 50–100 mL of ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated with a Kuderna–Danish steam evaporator for GC analysis.

For solid phase extraction, the C_{18} extraction cartridges were prepared by rinsing once with 2 mL of Resi-grade methanol and then twice with 3 mL of distilled water. The extraction efficiency of the C_{18} cartridge was determined by using [^{14}C]molinate (10.4 μCi/μmol), which was purified on Whatman LK5DF Linear-K Silica Gel TLC plates prior to use, with hexane–ethyl acetate (1:1) as the solvent system. Water spiked with [^{14}C]molinate and a suitable amount of unlabeled molinate to yield the desired concentration was passed through the cartridge. The cartridge then was serially eluted by centrifugation with 2-mL portions of 10%, 25%, 50%, and 75% aqueous methanol and pure methanol, and then with 1 mL of additional methanol. Each fraction was counted on a liquid scintillation counter (LKB Wallace 1217 Rackbeta).

Water samples (100–200 mL) were passed through a cartridge at a flow of about 15 mL/min under vacuum. The cartridges were next rinsed with ca. 3 mL of distilled water and then placed in a centrifuge tube where they were eluted with 2 mL and then five 1-mL portions of ethyl acetate or with two 2-mL portions of acetonitrile or methanol while centrifuging (500*g*) for 1 min. The organic solvent eluates were concentrated under nitrogen and

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adjusted to volume for GC analysis. For ELISA analysis, ethyl acetate extracts were transferred into a small volume of propylene glycol–acetonitrile (1:1) and the ethyl acetate was evaporated under nitrogen. The methanol or acetonitrile extracts were concentrated or diluted to a suitable volume (usually 0.5–10 mL) and used directly in ELISA analysis without using the propylene glycol–acetonitrile solvent mixture.

Extraction of Soil. Soil samples (20–50 g) were blended for 1 min with 250 mL of 20% (v/v) ethyl ether/methylene chloride. The mixture was filtered through Na_2SO_4 contained in a filter-paper-lined funnel. The residue was reblended with 200 mL of solvent for an additional minute. After again filtering through Na_2SO_4, and washing the blender 3 times with ethyl acetate, the organic phases were combined and concentrated in a Kuderna–Danish steam evaporator. These concentrates were analyzed by GC. For ELISA an aliquot of propylene glycol was added to an aliquot of the ethyl acetate. The ethyl acetate was evaporated from the mixture under a stream of nitrogen. An equal volume of acetonitrile was added to the propylene glycol, to decrease viscosity, and this mixture was analyzed. Soil samples that were spiked were allowed to stand at room temperature 30 min before beginning extraction.

Extraction of Air Samples. Air samples were collected on XAD-4 resin using an air sampler at a flow rate of 50 L/min as described in Seiber et al. (*11*) and Ross and Sava (*13*). The resin (ca. 50 mL) was shaken with separate portions of ethyl acetate for 30 min (100 mL), 20 min (70 mL), and 15 min (60 mL). The resulting solvent, sample bottle, and extraction flask washes were combined, concentrated in a Kuderna–Danish evaporator, and analyzed as described above for soil samples.

Gas Chromatography. A Hewlett-Packard Model 5710A GC equipped with an NP-TSD detector and a DB-5 capillary column, 30 m in length with film thickness 1.5 μm, was used. The column, injector, and detector temperatures were 170, 250, and 250 °C, respectively. The helium, air, and hydrogen flows were 1.5, 50, and 3 mL/min, respectively. The split ratio was 1:59. Quantitation was done relative to an external standard of molinate using peak areas determined with a Hewlett-Packard Model 3393A integrator.

ELISA Procedure. The procedure was similar to that previously described (*12*). Briefly, 96 well microtiter plates were pretreated with 0.02% glutaraldehyde. Each well was then coated with 200 μL of 2 μg of coating antigen/mL of 0.5 M carbonate buffer at pH 9.8 and incubated overnight at 4 °C. Meanwhile, the standard or sample was prepared by incubating molinate (3.9–2000 ng/mL) or the sample with the anti-molinate sera diluted 1/4000 with phosphate-buffered saline solution (pH 7.4) containing 0.05% Tween-20 and 0.02% sodium azide (PBS–Tween) at room temperature. The following day, the plates were washed 3 times with PBS–Tween, and then the standard- or sample–serum was added. After a 2-h incubation, the plates were washed as above, 200 μL of the goat-anti-rabbit IgG–enzyme conjugate (1/2000 diluted with PBS–Tween) was then added to each well and incubated for an additional 2 h. After the plate was washed, 200 μL of 1 mg/mL *p*-nitrophenyl phosphate in 10% diethanolamine buffer was added, incubated about 20 min, and the absorbance read at 405 nm in a Titertek Multiskan (Flow Laboratories). Two to four concentrations of each sample were routinely analyzed.

RESULTS AND DISCUSSION

Most extraction schemes for the analysis of pesticides, including for molinate in water by GC, require the use of relatively nonpolar organic solvents. Since such organic solvents have low solubility in buffer solution and can disrupt the antigen–antibody reaction, it is necessary to use polar and water-miscible solvents in ELISA analysis (*12*). Thus, a nonpolar extraction solvent must be exchanged to a water-miscible solvent, such as propylene glycol, prior to ELISA analysis. An attractive alternative is to use a solid-phase extraction column to remove the analyte from water and then to elute the column with a water-miscible solvent such as methanol or acetonitrile. The effects of methanol and acetonitrile–propylene glycol (1:1) on the ELISA standard curve for molinate are shown in Figures 1 and 2. There was no

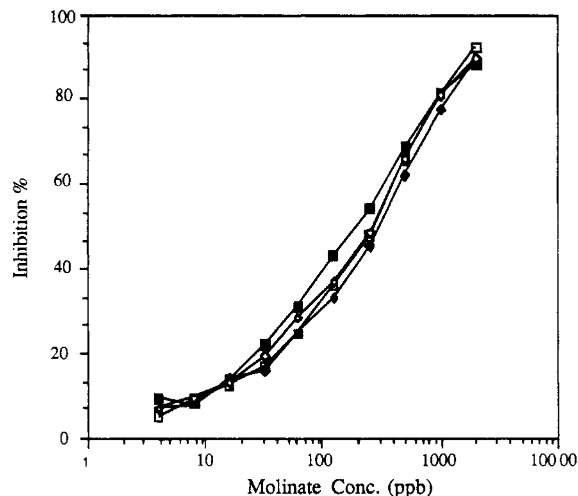

Figure 1. Effect of acetonitrile (ACN)–propylene glycol (PG) (1:1) on the ELISA standard curve for molinate: ACN/PG (■); 2% ACN/PG (◆); 5% ACN/PG (□); 10% ACN/PG (◇).

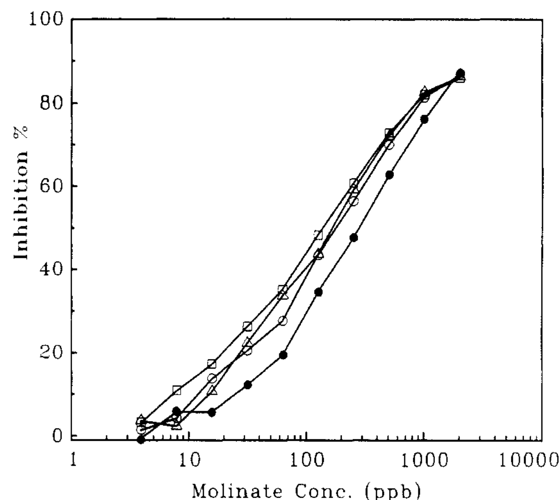

Figure 2. Effect of methanol (MeOH) on the ELISA standard curve for molinate: 0% MeOH (□); 2% MeOH (△); 5% MeOH (○); 10% MeOH (●).

significant effect when the ELISA buffer contained less than 10% (v/v) acetonitrile–propylene glycol (1:1) or 5% or less of methanol. At higher concentrations of these cosolvents, standard curves were significantly different ($p < 0.05$, Student's *t*) from buffer alone, being shifted to the right thus decreasing the detectability of the assay. In the experiments described here the total volume of organic solvent in the matrices was maintained at <5%. Figures 1 and 2 also show that the detection limit was about 15 ng/mL molinate in the buffer only samples, similar to that described in Gee et al. (*12*). It is important to note that antibodies will differ in their response to solvent. Some antigen/antibody interactions are very sensitive to solvents, while others are resistant, and the sensitivity of some assays can even be increased by the presence of some solvents (*12*).

Solid phase extraction was compared with a liquid–liquid partitioning procedure for spiked water using both GC and ELISA analysis. The apparent recoveries of spiked tap water at 10 and 100 ppb and 1 ppm measured by GC ranged from 99.7 to 103.5% for solid-phase extraction and 94.1 to 98.1% for liquid–liquid extraction. A similar agreement in apparent recoveries between the two extraction methods was also obtained by ELISA analysis (Table I) when the resulting ethyl acetate extracts were transferred into propylene glycol–acetonitrile (1:1). Further experiments were conducted with solid-phase extraction.

Table I. Recoveries of Molinate from Spiked Water Samples Measured by GC and ELISA

extraction procedure	water type	fortification concn, ppm	GC method % recovery[a]	ELISA method % recovery[a]	
				ACN–PG[b]	MeOH[c]
liquid–liquid	tap	0.01	95.7 ± 4.5	100.4 ± 6.9	
		0.10	98.1 ± 2.0	92.4 ± 6.5	
		1.0	94.1 ± 2.2	90.1 ± 3.8	
solid phase	tap	0.001	103.5 ± 1.9	96.3 ± 20.4	87.8 ± 9.5
		0.01	99.7 ± 2.1	91.7 ± 4.6	87.9 ± 15.6
		0.10	99.7 ± 4.9	90.6 ± 8.9	100.3 ± 11.1
		1.0	100.1 ± 1.4	93.6 ± 7.1	
	Putah Creek	0.01	96.0 ± 3.7	94.0 ± 10.6	
	drainage ditch	0.01	94.7 ± 0.8	96.7 ± 2.6	
	rice field	1.0	96.9 ± 3.2	111.0 ± 7.2	

[a] Average ± standard deviation of three sample replicates. [b] Samples were extracted in ethyl acetate and transferred into acetonitrile–propylene glycol (1:1). [c] Molinate was eluted from a solid phase extraction column with methanol. GC was done on the ethyl acetate eluate.

Table II. Percent Recovery of [^{14}C]Molinate following Elution from a Solid-Phase Cartridge[a]

% solvent in water	% recovery with methanol	% recovery with acetonitrile
10	0.7	1.3
25	1.9	5.1
50	9.2	29.7
75	53.2	54.4
100	33.2	5.6
100	1.4	0.7
total % recovery	99.7	96.7

[a] Water spiked with [^{14}C]molinate was passed through a C_{18} cartridge. Cartridges were eluted successively with the indicated solvent in water and fractions of the eluate counted by liquid scintillation counting (see Experimental Section).

In order to compare the ELISA method with the GC method, ethyl acetate was used to elute molinate from some C_{18} solid phase extraction cartridges. However, this necessitates a transfer of the ethyl acetate into propylene glycol–acetonitrile for ELISA analysis. Thus we examined the use of acetonitrile or methanol as solid phase column elution solvents for routine ELISA analysis. The elution profile is given in Table II and shows that aqueous acetonitrile and aqueous methanol were both effective eluting solvents, yielding cumulative molinate recoveries of 99.7% and 96.7%, respectively. These data show that complete recovery of molinate from the solid phase extraction columns will require the use of pure methanol or acetonitrile as eluting solvents. The use of a 3:1 (v/v) solvent–water mixture provides apparent recoveries greater than 50% and may be useful if the sample contains an interfering impurity not eluted with the 3:1 mixture.

Apparent recoveries of molinate from tap water spiked at 1, 10, or 100 ppb were 87.8–100.3% by ELISA when pure methanol was used to elute the cartridges compared with apparent recoveries of 90.6–96.3% when ethyl acetate was used. This compares favorably to the 99.7–103.5% apparent recovery by GC (Table I).

Several types of water that would normally be encountered when monitoring for molinate were spiked and analyzed by GC and ELISA. Turbid Putah Creek water and irrigation ditch water were spiked with 10 ppb of molinate. Rice field water was spiked at 1 ppm due to a high background molinate residue. Apparent recoveries were >90% by either GC or ELISA for all types of water (Table I). Thus, ELISA was able to detect 1 ppb molinate in spiked tap water and 10 ppb in local irrigation water or turbid Putah Creek water. The lower limit of 1 ppb could not be tested in Putah Creek water, as only 200 mL of water could be passed through the cartridge due to clogging of the cartridge by suspended particulate matter in this water. If the water were filtered prior to passing through the cartridge, this problem could potentially be bypassed.

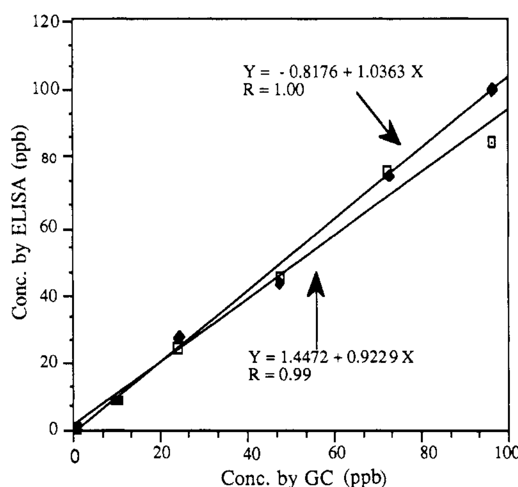

Figure 3. Correlation of GC vs ELISA for molinate analysis: ELISA with ACN/PG solvent transfer (□); ELISA with MeOH elution (◆).

Water samples from a rice field treated with molinate were analyzed by ELISA and GC to determine the correlation between the two methods. Cartridges were eluted with ethyl acetate and the eluate was split: one part was used directly for GC analysis, and a second part was transferred to acetonitrile–propylene glycol (1:1) for ELISA. A linear relationship was obtained with a slope of 0.923 and a correlation coefficient (r^2) of 0.986 (Figure 3). When methanol was used to elute the cartridges for ELISA analysis (for GC analysis, the cartridge was eluted with ethyl acetate), thus avoiding the solvent transfer step, the slope was 1.036 with r^2 of 0.995 (Figure 3). The methanol–ELISA method probably gave a better correlation since minimal evaporative steps or solvent transfer were needed.

To test the effect of soil extract on the ELISA, standard curves were run in the presence of extracts of untreated dryland soil. No effects were seen up to 5% soil extract; however, at the low end of the standard curve, data were more variable and the limit of detectability increased to 30 ng/mL.

Dryland soil samples spiked at 0.1, 0.5, and 1.0 ppm (three replicates each) were extracted and analyzed by GC and ELISA. Apparent recoveries were 82.4 ± 18.2, 76.4 ± 1.5, and 99.4 ± 9.4% for 0.1, 0.5, and 1.0 ppm, respectively, for ELISA and 108.3 ± 14.2, 99.5 ± 7.2, and 102.1 ± 5.3% for GC. The average relative standard deviations were 11.1% and 8.5% for ELISA and GC, respectively.

Table III shows the results of ELISA and GC analysis of several soil samples taken from a molinate-treated rice field.

Table III. Concentration of Molinate in Rice Field Soil Samples Analyzed by GC and ELISA

sample[a]	GC, ppm	ELISA, ppm
CK	0.019[b,c]	0.066[d]
5/19/85	0.162	0.298
5/20/85	0.186	0.401
5/22/85	0.480	1.442

[a] Soil samples were collected from a molinate-treated rice field. [b] Average of two injections of each sample extract. [c] A second aliquot of these soil samples was extracted and analyzed by GC only, concentrations were 0.014, 0.145, 0.196, and 0.496 for CK, 5/19/85, 5/20/85, and 5/22/85, respectively. [d] The same sample extract as used for GC analysis was analyzed by ELISA on two separate days. Each day each sample was run at three different dilutions in four well replicates.

Table IV. Concentration of Molinate in Air Samples from a Molinate-Treated Rice Field Analyzed by GC and ELISA

sample[a]	GC, μg/m^3	ELISA, μg/m^3
513MA	0.75[b]	0.67[c]
513MB	0.87	0.99
513MC	1.03	1.27
514MA	1.02	0.95
514MB	1.05	0.60
514MC	1.27	1.01
514WB	0.43	0.38
515MB	0.26	0.35
515MC	–[d]	0.32

[a] Air samples were collected as described in Seiber et al. (*11*). [b] Mean of two injections of the air sample extract. [c] The same sample extract used for GC was run in an ELISA at three dilutions and four well replicates for each dilution. [d] Sample was lost.

The correlation coefficient was 0.986. Although the correlation coefficient was good, ELISA values were generally up to 3 times higher than GC values. A matrix effect is possible since a different soil type had been used for recovery studies. Apparent recovery of spikes from the rice field soil sample was 84.0% and 160.5% by GC and ELISA, respectively. Sample extracts were concentrated to various volumes depending on the concentration of molinate. It appeared that in those samples containing higher levels of molinate (i.e. the soil extract was more dilute at the step of analysis), the ELISA value more closely approximated that of the GC. This is strong evidence for a matrix-derived interference.

To test the utility of the ELISA with air samples, XAD-4 resin was spiked with 0.1, 0.5, and 1 ppm molinate and then extracted and analyzed by GC and ELISA. ELISA-analyzed apparent recoveries were 94.3 ± 4.1, 97.7 ± 18.6, and 83.4 ± 7.6% for 0.1, 0.5, and 1 ppm, respectively. Apparent recoveries by GC were 103.1 ± 3.4, 87.5 ± 7.6, and 92.1 ± 3.1%. Average relative standard deviation was 10.8 and 5.1% by ELISA and GC, respectively. Table IV gives the concentration of molinate in several air samples from a treated field measured by GC and ELISA. A good correlation ($r = 0.785$) was obtained with a slope of 0.76.

For the data reported here the variability for ELISA was somewhat larger than for GC. Some of this error can be minimized by judicious selection and use of pipettors and microtiter plates (*14*). Some of the variables we tested are shown in Table V. The contributions to error as measured by the RSD (relative standard deviation) between channels of the pipettor or the spectrophotometer were less than 0.8%. When the plates were filled with dye alone, as a test of the plate reader and plates, a RSD of less than 2.4% was seen among plates, as well as among wells within the plate. Finally, the plates were coated with antigen, and the same sample–antibody solution (60 ng/mL of molinate) was added to each well. The RSDs among plates and wells within the plate were less than 8.0%. This indicates that the main source of variability is due to the efficiency and uniformity of coating antigen binding or the antigen–antibody reactions.

Table V. Some Sources of Error in the ELISA Procedure

variable	rel std dev, %
plates/wells	<2.4
coating antigen	<8.0
spectrophotometer	<0.35
pipettor A[a]	
between channels	<0.78
between pipettings	<0.40
pipettor B[a]	0.60

[a] Pipettor A was a Flow Titertek 12-channel pipettor. Pipettor B was a Rainin Pipetman, Model P-200.

CONCLUSIONS

ELISA methodology showed a good correlation with a conventional GC method for spiked or field samples of water, soil, and air. In a previous study the same ELISA system was used for the analysis of rice field water samples without extraction, and this was compared to the same samples extracted and analyzed by GC (*15*). Correlations obtained by Harrison et al. (*15*) were identical with those obtained here, in which the samples were extracted for both ELISA and GC analysis. Sample throughput time is obviously decreased in the case of Harrison et al. (*15*), where the samples were analyzed without workup.

The indirect ELISA can be coupled with solid phase extraction to yield a sensitive and selective analytical method for molinate in water to a demonstrated detection limit of 1 ppb. Although sample workup time may not be improved by using solid-phase extraction, total sample throughput time is improved due to the large number of samples that can be processed in parallel. This parallel processing is particularly amenable to automation. For example, a fully automated ELISA system, which is used to screen for plant pathogens, is capable of processing 10000–15000 samples per day (*16*).

The solid phase extraction method appears simple, precise, and fast when applied to water samples. Either acetonitrile or methanol can be used to elute the cartridge for routine ELISA analysis, bypassing the need for solvent exchange and volume reduction needed with liquid–liquid extraction. GC generally provided better precision (1–5% standard deviation) then did ELISA (3–20% standard deviation). We believe that the ELISA precision can be improved substantially by improving the uniformity of the coating antigen. Similarly improvements in sensitivity, by altering the format, would possibly allow us to dilute out the matrix interference observed for some soil samples, while still being able to quantitate the molinate. However, for most studies of the fate of molinate in rice field water and surrounding air, the precision provided by the ELISA method described in this paper is acceptable, particularly when combined with the possible major time advantage of sample throughput provided by ELISA.

Registry No. Molinate, 2212-67-1; water, 7732-18-5.

LITERATURE CITED

(1) Van Emon, J.; Seiber, J. N.; Li, Q. X.; Hammock, B. D. "Immunoassay Techniques for Pesticide Analysis". In *Analytical Methods for Pesticides and Plant Growth Regulators*: *Modern Analytical Techniques and Specific Applications*; Zweig, G., Sherma, J., Eds.; Academic Press: New York, in press; Volume XVI.
(2) Vanderlaan, M.; Watkins, B. E.; Stanker, L. *Environ. Sci. Technol.* **1988**, *22*(3), 247–254.
(3) Newsome, W. H. *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 9–14.
(4) Kelley, M. M.; Zahnow, E. W.; Petersen, W. C.; Toy, S. T. *J. Agric. Food Chem.* **1985**, *33*, 962–965.
(5) Huber, S. J.; Hock, B. Z. *Pflanzenkrankheiten Pflanzenschutz* **1985**, *92*, 147–156.

(6) Newsome, W. H. *J. Agric. Food Chem.* **1985**, *33*, 528–530.
(7) Van Emon, J.; Hammock, B.; Seiber, J. N. *Anal. Chem.* **1986**, *58*, 1866–1873.
(8) Huber, S. J. *Chemosphere* **1985**, *14*, 1795–1803.
(9) Wie, S. I.; Hammock, B. D. *J. Agric. Food Chem.* **1982**, *30*, 949–957.
(10) Cornacchia, J. W.; Cohen, D. B.; Bowes, G. W.; Schnagl, R. J.; Montoya, B. L. *Rice Herbicides, Molinate (Ordram) and Thiobencarb (Bolero): A Water Quality Assessment*; California State Water Resources: Sacramento, CA, 1984.
(11) Seiber, J. N.; McChesney, M. M.; Sanders, P. F.; Woodrow, J. W. *Chemosphere* **1986**, 15, 127–138.
(12) Gee, S. J.; Miyamoto, T.; Goodrow, M. H.; Buster, D.; Hammock, B. D. *J. Agric. Food Chem.* **1988**, *36*, 863–870.
(13) Ross, L. J.; Sava, R. J. *J. Environ. Qual.* **1986**, *15*, 220–225.
(14) Harrison, R. O.; Hammock, B. D. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 981–987.
(15) Harrison, R. O.; Gee, S. J.; Hammock, B. D. In *Biotechnology for Crop Protection*; ACS Symposium Series Vol. 379, Hedin, P., Menn, J. J., Hollingworth, R. M., Eds.; American Chemical Society: Washington, DC, 1988; pp 316–330.
(16) van Vuurde, J. W. L.; Maat, D. Z.; Franken, A. In *Biotechnology for Crop Protection*; ACS Symposium Series Vol. 379, Hedin, P., Menn, J. J., Hollingworth, R. M., Eds.; American Chemical Society: Washington, DC, 1988; pp 338–350.

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Design Principles for a Large High-Efficiency Sub-Boiling Still

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The sub-boiling method of acid purification for low trace element blank has now been in use for about 20 years. However, to achieve commercially useful yields, distillates must be produced at ~25–100 L/day. Aspects of still design that include throughput have been examined, including variables such as condenser area, temperatures, distance between the condenser and the liquid in the still pot, and coolant temperature. Designs for several new stills are given and compared to those of prior sub-boiling stills. Perfluoroalkoxy resin construction was used both for ease of fabrication and for the distinct distillate purity.

High-purity acids are of fundamental importance in analytical laboratories for achieving low blanks in trace metals analysis schemes. Sub-boiling distillations are now commonly used to purify acids. Recently, we have atempted to improve sub-boiling distillate purity by increasing throughput. Following are some other reasons for designing a large, high-throughput sub-boiling still.

First, a number of industries have need of very high purity reagents in quantities of hundreds of liters per day. This exceeds present sub-boiling capabilities by 2 or more orders of magnitude. For example, for volatile acids, most sub-boiling stills can produce 0.5–1 L/day whereas industrial users consume lots of 55-gal (~210 L) drums. Industrial users also need a cost per liter considerably less than can be achieved with the present sub-soiling stills. A high-throughput still could improve supply and cut costs.

Second, improved reagent quality should result, in principle, from an increase in yield per unit time per unit area of the condenser since the condenser is the only part of the still other than the distillate receiver that is in contact with the distillate. If improved distillate quality does not result, it would be another indication that trace metal impurities are derived primarily from contact with the container wall.

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NIST (formerly NBS) has employed sub-boiling distillation from quartz and Teflon TFE (tetrafluoroethylene) stills since 1971 as a means of purifying reagent acids, water, and some solvents (*1*, *2*). Mattinson proposed a device in 1971 for the same purpose, which can be described as a "two-bottle still" (*3*). Other proposals for stills have been advanced from time to time, and there are now at least four commercial sources of sub-boiling stills worldwide.

All of these products are close analogues of the design of the Quartz et Silice (Paris, France, Model PB-5, PB-10, or PB-15) quartz still or the original NBS design Teflon TFE still (*1*). All of the commercial products of which we are aware appear to produce distillates of very high purity with respect to trace metal impurities. Despite considerable effort to improve distillate purity at NIST, only the analysis of the distillates has improved while the actual reagent purity has remained unchanged. This fact and other evidence have implicated the container of the distillate as the limiting factor (*4*, *5*).

We have constructed a series of three new Teflon PFA (perfluoroalkoxy) stills of new design, which have the capability of distilling up to ~45 L/day. We have met our goals of producing equivalent purity acid at distillation rates of 10–50 times higher than that of the previous design. The design principle that we implemented will be discussed, and the distillation of reagents such as HCl, HF, HNO_3, and H_2O will be described.

Apparatus. *Still 1.* This unit was fabricated by the Fluoroware Corp. from a 30-gal (115 L) Teflon PFA drum liner. The general dimensions and internal configuration of the still are given in Figure 1, which is based upon a scale-up of the NBS Teflon TFE still (*1*) shown in Figure 2. Both bulkheads are penetrated by welded fittings to permit access to the internal parts of the still. Heating elements were provided both in the headspace and in the still pot reservoir.

The heating elements were fabricated from nichrome wire wound on a quartz rod and electrically insulated from a stainless steel tube sheath. This was slid into a Teflon PFA tube and screwed into the bulkhead as indicated. Power to the heating elements was controlled by variable transformers,

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