Bull. Environm. Contam. Toxicol. 29, 469–475 (1982)

Evaluation of Maintenance Conditions for Plants Prior to Pesticide Residue Analysis

Stella D. Elakovich

Department of Chemistry, University of Southern Mississippi, Hattiesburg, MS 39406

Extensive surveys of pesticide residues in the environment (sponsored by the National Pesticide Monitoring Program, see CAREY et al. 1979, for example), studies of field techniques (AMBRUS 1979), pesticide persistence and metabolism (STEINWANDTER 1978; FUHREMANN and LICHTENSTEIN 1980; and many others), pesticide extraction techniques (WHEELER et al. 1978) and pesticide detection methods (VOLLNER and KORTE 1980) have been reported. All these studies require storage of samples from the time of collection until the analyses. The time lag from collection to analyses may range from hours to months.

This paper evaluates six methods of maintaining plant samples after collection until analyses and examines the effects of these methods on the amount of recovered pesticide residue. The study is limited to the relatively stable pesticides lindane and methoxychlor and to a single field-grown plant species of high moisture content and high summer growth rate. The maintenance conditions were the following six procedures:

1) freshly collected plants (fresh)
2) plants frozen immediately upon collection (frozen)
3) plants dried at room temperature and ground to 20 mesh size (RT-20)
4) plants dried at room temperature and ground to 40 mesh size (RT-40)
5) plants dried at 56°C and ground to 20 mesh size (56°-20)
6) plants dried at 56°C and ground to 40 mesh size (56°-40)

MATERIALS AND METHODS

Plant Selection. A homogeneous population of Alternanthera philoxeroides (alligator weed), an aquatic plant with 84% total moisture and a known July productivity of over 12 kg/m^2 (WOOTEN and SCHEETZ 1980), was selected as the test species. The high moisture content of alligator weed should serve to exaggerate any differences caused by examination of fresh, fresh-frozen or dried plant materials. High biomass productivity during the period from pesticide application to sample collection should increase the rate of uptake of aerially applied pesticides.

Pesticide Selection. Lindane and methoxychlor were chosen as the test pesticides because of their proven long-term stability

0007-4861/82/0029-0469 $01.40

(see WALISZEWSKI 1980 and PERUMAL et al. 1979), their availability for public use, and their gas chromatographic elution behavior. Lindane, the more volatile, elutes rapidly from an OV-17 column, whereas methoxychlor elutes late in a series of common pesticides. Analytical samples of both lindane and methoxychlor were obtained from Supelco, Inc., Bellefonte, PA; compounds for field application were the commercial formulations of Kill-Ko from Rigo Company, Bruckner, Kentucky, which contained 20% of the γ-isomer of lindane, 38.2% naphtha and 41.8% inert materials, and of Marlate-50 from E.I. duPont de Nemours and Company, Inc., Wilmington, DE, which contained 44% of 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane (methoxychlor), 6% other isomers and 50% inert materials.

Extraction Solvents. Hexane, isooctane and acetonitrile were purchased pesticide grade solvents. Chloroform and methanol were glass distilled in our laboratories and checked for purity by gas chromatography.

Chromatographic Analysis. Samples were subjected to gas chromatographic (gc) analysis on a Perkin Elmer Sigma 1 gc equipped with an electron capture detector and an OV-17 capillary column under 200°C isothermal conditions. Samples were analyzed in triplicate except in those cases where duplicate injections differed by less than two percent. Calibration curves for lindane, methoxychlor and aldrin were prepared under the same conditions.

Pesticide Application and Sample Collection. An approximately 6' x 6' test site of a homogeneous population of Alternanthera philoxeroides was delineated and plants were treated with a mixture of commercial formulations of lindane and methoxychlor, diluted according to manufacturer directions. Spray was applied with a continuously agitated hand-held sprayer. Eighteen days following pesticide application, six subsample 0.5 m^2 quadrats of the treated plants, including both above- and belowground parts, were collected and immediately placed in ice chests for transportation to the laboratory. The plants were thoroughly rinsed with tap water to remove adhering soil and were air dried to the drip-dry state. Two 100 g and two 250 g aliquots were taken from each of the six subsample quadrats. One 100 g aliquot from each of the six quadrats was processed immediately as a fresh plant sample; the second 100 g aliquots from each were frozen. One 250 g aliquot from each quadrat was placed in a 56°C drying oven; the second 250 g aliquot from each was dried in a plant dryer. The maximum temperature in the plant dryer was 42°C; the upper layer of plants were not this warm as the heat was from the bottom. In this paper, plants dried in the plant dryer are referred to as room temperature (RT) dried plants. The separate aliquots of dried plants were weighed and ground to 20 mesh size in a Wiley mill. Each ground sample was thoroughly mixed to achieve homogeneity and a sample equivalent to a 100 g wet-weight sample was removed from each for analysis. The remaining 20 mesh size ground material was reground to 40 mesh size, and a sample equivalent to a 100 g wet-weight sample was removed for analysis.

Pesticide Extraction. To insure complete pesticide residue extraction from the samples, the methanol-chloroform 12 hour continuous Soxhlet extraction method of MUMMA et al.(1966) was employed. Sample work-up followed the method described in the FDA Pesticide Analytical Manual (1977); samples were cleaned-up with Florisil as described in this reference. A 50 mg sample of aldrin was added to each Soxhlet thimble prior to extraction as an internal standard. Samples were concentrated to 10.0 ml and then diluted 1:10. Concentrations were measured as ng/μL.

Data Analysis. The results of the experiments were analyzed separately using a Sigma 9 computer and a Statistical Package for the Social Sciences (NIE et al. 1975). The data are uncorrected. Statistical analyses included: 1) Bartlett-Box F for homogeneity of variances of all treatments, 2) two-way analyses of variances only for data from dried materials which involved two mesh sizes and two temperatures; recovered pesticides from fresh and frozen materials were from single types of treatments, 3) for data sets showing no first order interactions, one-way analyses of variances for recovered pesticides from dried materials and the Duncan Multiple Range test for differences between all treatment means, and 4) for data sets showing first order interactions, two-tailed t-test of the differences between means of all treatments, two at a time (SOKAL and ROHLF 1969).

RESULTS AND DISCUSSION

Preliminary Data Analyses. Bartlett's test indicated equality of variance in the groups of samples. A two-way analysis of variance showed no significant ($P > 0.05$) interaction between temperature and mesh for recovered lindane (TABLE 1), so the Duncan Multiple Range Test and one-way ANOVA were used for significance testing. Interaction of temperature and mesh for recovered methoxychlor was statistically significant (TABLE 1), so two-tailed t-test were performed on the means of all treatments.

Lindane. Mean of recovered lindane from freshly collected plants (fresh) was largest, had the highest standard deviation and differed significantly from means of all other treatments (TABLE 2).

TABLE 1. Two-way analysis of variance of recovered pesticides from dried materials.

	Lindane conc.			Methoxychlor conc.		
Source	df	F	P	df	F	P
temperature	1	6.953	*	1	22.567	***
mesh	1	12.431	***	1	5.102	*
temp X mesh	1	1.151	NS	1	6.8	*
error	67	(0.0284)+		63	(2.29)+	

NS = $P > 0.05$; * = $0.05 \geq P \geq 0.01$; ** = $0.01 \geq P \geq 0.001$; *** = $P < 0.001$.
+ = error mean square

TABLE 2. Results of Multiple Range Test on means of lindane concentration by treatment. (Means connected by lines are not significantly different at the .05 level.)

Treatment	Lindane ave. conc. (ppm)	(S.D.)	Number of Samples
56°-40	.281	(±.175)	18
RT-40	.344	(±.093)	18
56°-20	.380	(±.181)	18
frozen	.492	(±.263)	18
RT-20	.529	(±.207)	17
fresh	1.137	(±.452)	12

Means of treatments RT-20 and RT-40 differed significantly using both the Duncan Multiple Range test and one-way analysis of variance (F_s = .0016) (Figure 1). This is in contrast to means of treatments 56°-20 and 56°-40 which did not differ significantly (TABLE 2, Figure 1). Mean of recovered lindane from frozen material did not differ significantly from means of treatments ground to 20 mesh and dried at RT or 56°, but was significantly different from means of RT-40 and 56°-40 treatments (TABLE 2). These results were not expected.

The smallest amount of lindane was recovered from plants oven dried at 56° and ground to 40 mesh size (56°-40). This is the expected result when the reported volatility of lindane is considered. For example, AHMED and AUSAF (1978) found that 50% of lindane applied as dust to chick-peas is lost over a 2 month period at 32°

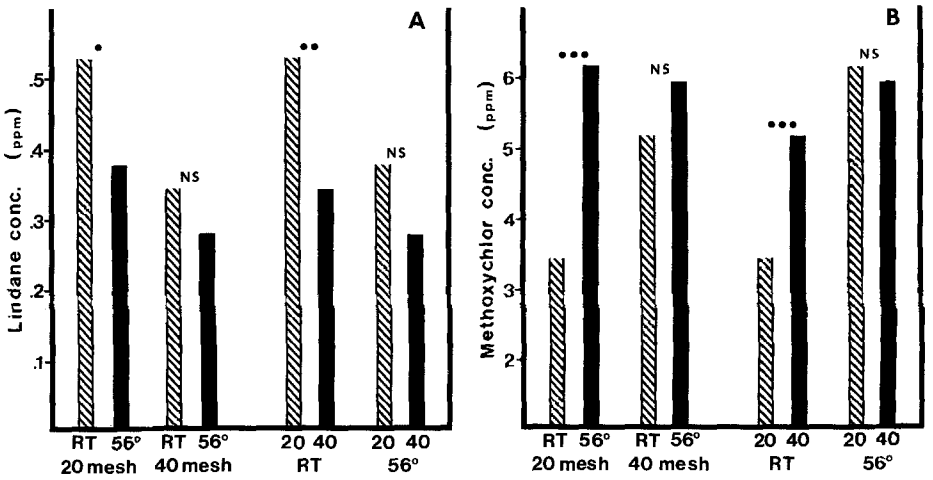

Figure 1. (A) Results from one-way ANOVA for means of recovered lindane from dried samples (f probability: NS ≥ .05, • = f probability < .05, •• = f probability < .01). (B) Results of two-tailed t-test on means of recovered methoxychlor from dried samples (NS= not significant, ••• = P < .001).

storage temperatures. EICHLER (1976) was able to show that more than 50% of lindane sprayed onto tomato plants had evaporated within one week. PERUMAL et al. (1979) found that lindane could be safely stored (before use) for six months during which time it loses less than three percent of its activity; after 24 months only 8.3 percent of activity is lost. Thus, lindane is stable but volatile.

Graphed means of recovered lindane concentration from the samples that were dried and results of one-way analysis of variance are shown in Figure 1A. Room temperature treatments yield higher lindane concentrations than 56° treatments, although the differences in RT-40 and 56°-40 are not significant at $\alpha = .05$. These results again reflect the volatility of lindane discussed above. Grinding to 40 mesh size appears to decrease the amount of lindane recovered. With room temperature drying, the reduction is greatest; drying at 56° reduces the differences due to mesh size until they are no longer significant statistically ($\alpha = .05$), even though they are obvious from Figure 1A. Since one would expect that more finely ground particles would allow more efficient extraction, the above results are surprising. If one assumes that the extraction method used is so efficient that 20 mesh is sufficiently small to allow complete extraction of all lindane present, then an explanation for lower recoveries at 40 mesh must be found. It is possible that some lindane is lost during the grinding process due to heat generated by the Wiley mill. HORWITZ (1979) emphasizes that the decision of how fine to grind samples prior to analysis requires a balance between the difficulty of grinding and the changes produced by the heat and aeration of the grinding process. Since lindane is volatile, loss due to heat from grinding is possible although excess heat was not noticeable during the process. Another possibility is that a smaller mesh size produces a larger surface area available for water absorption, and since samples were weighed after grinding, a given 40 mesh size sample had more absorbed water and therefore less plant material and less lindane. However, since yields of methoxychlor were greater for RT-40 treatments, this second explanation seems unlikely.

Methoxychlor. There is interaction between mesh size and drying temperature (TABLE 1). Both 56° drying and 40 mesh size yield higher amounts of recovered methoxychlor, in striking contrast to the lindane results (Figure 1A, B). At 20 mesh size RT and 56° treatment means differ significantly ($P < .001$), indicating that drying temperature is important (TABLE 3). At 40 mesh size, the temperature effect is swamped out and means for recovered methoxychlor from RT and 56° treatments are not significantly different. For RT dried samples, recovered concentration means from 20 and 40 mesh sizes differ significantly ($P < .001$) indicating mesh size is important, whereas at 56° the mesh size effect is swamped out (NS). The mean for recovered methoxychlor from RT-20 samples (3.46 ± 0.72 ppm) is significantly smaller and the significance higher ($P \leq .001$) compared to all other treatments except fresh (NS) (TABLE 3). The mean for recovered methoxychlor from fresh samples (3.75 ± 0.99 ppm) is also significantly smaller (at least $P < .01$) than that for the remaining four treatments. The means of recovered methoxychlor

from these remaining four treatments, frozen, RT-40, 56°-20 and 56°-40, are not significantly different ($P > .05$). Thus one is forced to conclude that, for methoxychlor, lower recoveries are achieved if samples are analyzed immediately (fresh) or are dried at room temperature and ground to 20 mesh (RT-20). A rationale for this behavior is not immediately obvious.

TABLE 3. Means of methoxychlor concentration, standard deviations, and matrice of comparison between treatment means using paired t-test.

Treatment	Mean	(S.D.)	frozen	RT-20	RT-40	56°-20	56°-40
fresh	3.75	(±0.99)	**	NS	**	***	***
frozen	5.65	(±2.01)		***	NS	NS	NS
RT-20	3.46	(±0.72)			***	***	***
RT-40	5.18	(±1.77)				NS	NS
56°-20	6.18	(±1.55)					NS
56°-40	5.95	(±1.71)					

NS = not significant; * = $P < .05$; ** = $P < 0.01$; *** $P < 0.001$

CONCLUSIONS

The original question posed by this investigation was 'Does sample treatment affect the amount of pesticide recovered?' The answer is clearly 'yes'. Mesh size can change the amount of pesticide recovered; sample drying temperature can change it; whether plant samples are dried or not can change it. The degree and direction of change depend on the pesticide being examined. The results of this limited study suggest that the best sample maintenance method is the compromise one that plant samples be frozen from collection until analysis time.

ACKNOWLEDGEMENTS

Thanks are due to Dr. J. W. Wooten for helpful discussions and data analysis, to Ms. G. Megason for technical assistance, and to EPA Cooperative Agreement No. 80805501 for financial support.

REFERENCES

AHMED, H. and R. AUSAF: Pakistan J. Sci. Ind. Res. 21, 78-80 (1978).

AMBRUS, A.: International Congress of Pesticide Chemistry, 4th Ed. Advances in Pesticide Science, Pergamon Press, 620-632 (1979).

CAREY, A.E., J.A. GOWEN, H. TAI, W.G. MITCHELL, and G.B. WIERSMA: Pestic. Monit. J. 12, 209-229 (1979).

EICHLER, D.: Lindane, Proceedings Symposium, 84-101 (1976).

FUHREMANN, T.W. and E.P. LICHTENSTEIN: J. Agric. Food Chem. 28, 446-452 (1980).
HORWITZ, W.: International Congress of Pesticide Chemistry, 4th Ed. Advances in Pesticides Science, Pergamon Press, 649-655 (1979).
MUMMA, R.O., W.B. WHEELER, D.E.H. FREAR, and R.H. HAMILTON: Science 152, 530 (1966).
NIE, N.H., C.H. HULL, J.G. JENKINS, K. STEINBRENNER, and D.H. BENT: Statistical Package for the Social Sciences, 2nd Ed. New York, McGraw Hill Book Co. (1975).
PERUMAL, R., C.P. RAJ, A. CHANDASEKARAN and K. GOVINDARARAJ: Food and Farming Agric. 11, 17-19 (1979).
Pesticide Analytical Manual, Volume I, Methods which Detect Multiple Residues, U.S. Department of Health, Education and Welfare, Food and Drug Administration, Aug. 1 (1977).
SOKAL, R.R. and F.J. ROHLF: Biometry. San Francisco, W.H. Freeman and Company (1969).
STEINWANDTER, H.: Bull. Environ. Contam. Toxicol. 20, 535-536 (1978).
VOLLNER, L., and F. KORTE: Intern. J. Environ. Anal. Chem. 7, 191-204 (1980)
WALISZEWSKI, S.: Mater. Ses. Nauk. Inst. Ochr. Rosl. (Poznan) 20, 249 (1980).
WHEELER, W.B., N.P. THOMPSON, P. ANDRADE and R.T. KRAUSE: J. Agric. Food. Chem. 26, 1333-1337 (1978).
WOOTEN, J.W. and R.W. SCHEETZ: Studies of Emergent Aquatic Plants as Renewable Energy Sources. Final Report submitted to the Department of Energy, Solar Energy Research Institute, Oct. 3 (1980).

Accepted August 8, 1982