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Note

Facile gas chromatographic method for the determination of residues of Bidrin in pecan*

PROMODE C. BARDALAYE* and WILLIS B. WHEELER
Pesticide Research Laboratory, Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611 (U.S.A.)
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Bidrin or dicrotophos[1,2], the chemical name of which is dimethyl-(*E*)-2-dimethylcarbamoyl-1-methylvinyl phosphate according to IUPAC or (*E*)-3-(dimethylamino)-1-methyl-3-oxo-1-propenyl dimethyl phosphate according to *C.A.* nomenclature, is a contact and systemic insecticide approved in the U.S. for registered use primarily to control several insecticidal and acaricidal adversaries in cotton. It is also effective in control of elm bark beetle and coffee borer. Like other agrichemicals, the organophosphorus insecticide is highly beneficial to crops, but is also extremely toxic[3,4] to fish, birds and other wild life. Organophosphate poisoning resulting in death of a wide population of birds due to misuse of dicrotophos (and monocrotophos) in Texas in 1982 were reported[5]. Organophosphates inhibit acetylcholinesterase in the nervous system whereby synaptic transmission of nerve impulses are disrupted. Death usually occurs from asphyxiation because of failure of the respiratory center of the brain[6]. The gastrointestinal tract of the dead birds contained residues[5] of dicrotophos at a level of 5.6–14 mg/kg.

Recently there has been great interest in Bidrin for use in pecan cultivation to control pecan aphid bud moth, phylloxera and webworms. It is, therefore, desirable to conduct experimental field trials representative of different geographical areas of the U.S. to generate data for tolerance and registration purposes. Consequently, a need exists for validation and/or development of an adequate analytical methodology for quantitative assessment of the insecticide residues in this particular commodity.

Residue analysis of Bidrin in several substrates employing gas–liquid chromatography (GLC) with flame photometric detection is known[7–11]. However, Bidrin residue analysis in pecans is relatively unknown, and hence the present research was undertaken to develop an analytical method to determine Bidrin in pecan at various stages of treatment and/or use pattern. It is through modification and refinement of an earlier technique[11] that the procedure reported herein has been developed.

MATERIALS AND METHODS

Field trial and Bidrin application

An experimental protocol was developed by IR-4, the U.S. National Agricul-

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tural Program for clearance of pesticides, animal drugs, microbials and biochemicals for minor or special uses. It consists in employing 2 ml of the formulation Shell Bidrin 8 water miscible insecticide (EPA Reg. No. 201-274; contains 82% active ingredient by weight, or 2 lbs. of active ingredient per quart) diluted to 2 l with water. The pecan trees were grown on plots adequate to reflect actual commercial use conditions, each plot having four replicates. The insecticide was applied by low-pressure trunk injection of 2 ml of the diluted material prepared as above per 6 in. trunk circumference of the pecan trees. Applications were restricted to a minimum of one and a maximum of two per season beginning at early bud break stage and/or at half leaf maturity. In addition, the insecticide was not applied within 120 days of harvest. Samples for residue analysis were collected at harvest from trees receiving one application at early bud break, and from those receiving one application at half leaf maturity, and also from those receiving applications at both early bud break stage and half leaf maturity.

Preparation, extraction and cleanup of samples

Soon after harvest the pecans were shelled, and the nuts and shucks were stored at −10°C. Before analysis, they were thawed and chopped. Representative 25-g samples of the crop (nuts and shucks) were extracted with 100 ml methanol in a Polytron Homogenizer for 5 min at medium speed. The contents were then centrifuged. Aliquots of 10 ml of the supernatant representing 2.5 g crop were transferred to 120 × 10 mm PTFE-lined, screw-capped test tubes followed by addition of 0.5 ml water. After reducing the volume of the mixture to *ca.* 0.7 ml by a gentle stream of nitrogen, 4.5 ml water and 5 ml hexane were added to the concentrated aliquot. The contents were thoroughly shaken and centrifuged for complete separation of aqueous and organic layers. The hexane phase was discarded, and the washing of the aqueous phase was repeated with another volume of 5 ml hexane. The layer was again discarded, and the aqueous layer was partitioned thrice with 5 ml chloroform each time. The chloroform layers were pooled while the aqueous layer was finally discarded. The chloroform extract thus obtained was concentrated almost to dryness using a gentle stream of nitrogen, and the residue was dissolved in 1 ml ethyl acetate. Aliquots of 5 μl were used for GLC.

Reagents

All organic solvents were pesticide grade or HPLC grade. Bidrin of 99.0% purity was obtained from the Environmental Protection Agency, U.S.A.

Gas–liquid chromatography

A Hewlett Packard 5730A gas chromatograph equipped with a flame photometric detector (phosphorus mode) and a 1.2 m × 4 mm I.D. glass column packed with 1% Reoplex 400 on Gas-Chrom Q, 100–120 mesh, were used. The operating parameters were: injector port, 200°C; column, 170°C; and detector, 200°C. The column flow was 60 ml/min of nitrogen, and the air, hydrogen and oxygen flow-rates to the detector were regulated at 80 ml/min, 200 ml/min and 30 ml/min, respectively.

Fortification procedure

Representative 25-g samples of finely chopped crop demonstrated not to con-

tain any interferences or residues of Bidrin were fortified with known volumes of working standard solutions to give 0.01–0.5 mg/kg level of Bidrin. The fortified samples were mechanically shaken, and allowed to stand for *ca.* 1 h at room temperature before extraction. The entire constants were extracted without any subsampling.

RESULTS AND DISCUSSION

Bidrin was found to be eluted at 2.38 min in GLC, and hence the determinative step did not take more than 5 min per sample. The limit of detection was found to be 0.01 mg/kg. Typical chromatograms obtained from standard, control and fortified pecan are shown in Fig. 1. Recoveries ranged from 92 to 98% at fortification levels of 0.01–0.5 mg/kg (Table I). No residues of Bidrin were found in pecan harvested 120 days or longer after Bidrin treatment.

Fresh, untreated, finely chopped samples were fortified with standard solution of Bidrin, and subjected to the same treatment as the samples as a check on the analytical procedure, and any apparent loss or degradation during storage. No conceivable breakdown was observed under the conditions employed.

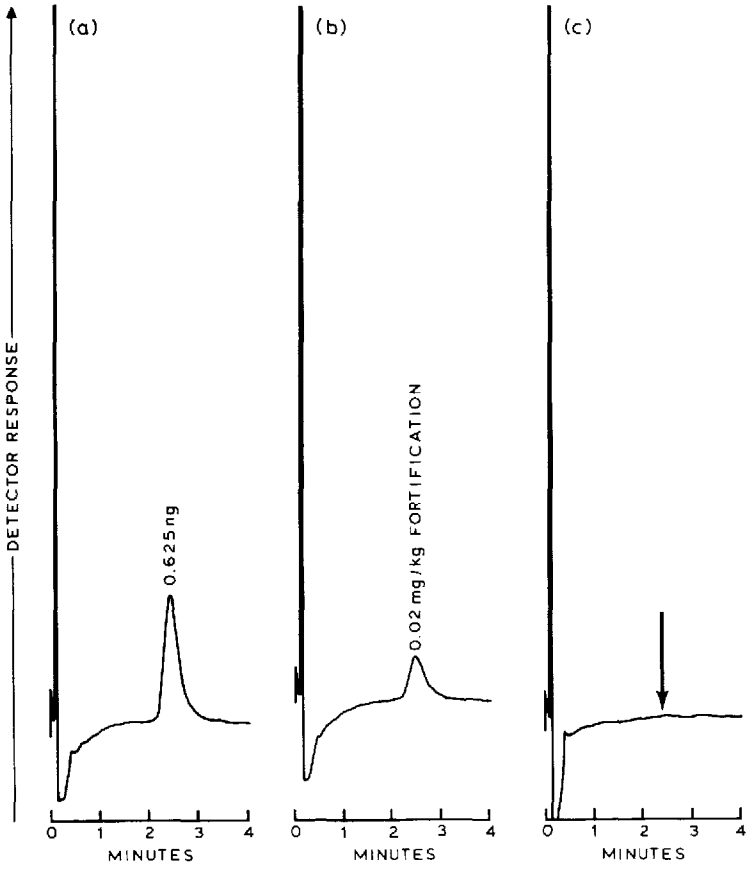

Fig. 1. (a) Chromatograms of (a) standard Bidrin (0.625 ng), (b) pecan control sample fortified with Bidrin (0.02 mg/kg) and (c) pecan control sample. See text for chromatographic conditions.

TABLE I

RECOVERY OF BIDRIN FROM PECAN

Values represent the average of three replicate determinations with standard deviations.

Fortification level (mg/kg)	*Recovery (%)*
0.01	96 ± 5.2
0.02	94 ± 4.1
0.05	98 ± 5.3
0.10	98 ± 4.8
0.50	93 ± 5.6

According to these results, residues of Bidrin in pecan at harvest 120 days or longer following treatment with the insecticide at the given rate would be less than 0.01 mg/kg. In addition, translocation from the treatment sites would be so low as to be undetectable. Because of its low application rate and residue contents in pecan, Bidrin should cause minimal environmental and food contamination. However, residue analysis in soil and run-off water, and studies concerning any possible degradation of Bidrin to other compounds are needed for proper environmental impact and food toxicity assessment.

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REFERENCES

1 G. L. Berg (Editor), *Farm Chemicals Handbook*, Meister Publishing Co., Willoughby, 1984.
2 *The Agrochemicals Handbook*, The Royal Society of Chemistry, Nottingham, 1983.
3 R. K. Tucker and D. G. Crabtree, *U.S. Fish Wildlife Serv. Resour. Publ.*, 84 (1970) 21–29.
4 W. T. Hill, R. G. Heath, J. W. Spann and J. D. Williams, *U.S. Fish Wildlife Serv., Spec. Sci. Rep., Wildlife*, 91 (1975) 11–12.
5 E. L. Flickinger, D. H. White, C. A. Mitchell and T. G. Lamont, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 827 828.
6 E. F. Hill and W. J. Fleming, *Environ. Toxicol. Chem.*, 1 (1982) 27–38.
7 W. Krijgsman and C. G. van de Kamp, *J. Chromatogr.*, 117 (1976) 201–205.
8 *Pesticide Analytical Manual*, Food and Drug Administration, Washington, DC, 1985, Pesticide Reg. Section 180.299.
9 K. A. Ramsteiner and W. D. Hörmann, *J. Chromatogr.*, 104 (1975) 438–442.
10 G. Zweig and J. Sherma, *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. VI, Academic Press, New York, 1972, pp. 287–298.
11 M. C. Bowman, *J. Agric. Food Chem.*, 15 (1967) 465–468.