

Committee on Residues and Related Topics

Pesticides and Other Chemical Contaminants

DAVID SODERBERG

U.S. Environmental Protection Agency, OPP, HED, RRB3, Rm 821D, Crystal Mall II, 7509C, Ariel Rios Bldg, 1200 Pennsylvania Ave, Washington, DC 20460, Tel: +1-703-308-4137, Fax: +1-703-305-5147, E-mail: soderberg.david@epamail.epa.gov

Summary

One new topic needs to be added to this refereeship this year. This topic is *Pesticides in Foods Using Acetonitrile Extraction and Partitioning with Magnesium Sulfate*. Method **2002.05**: The topic, *Determination of Glyphosate and Aminomethylphosphonic acid (AMPA) in Crops*, has been declared an Official Final Action Method (*Inside Laboratory Management*, May/June 2003). In the same issue of *Inside Laboratory Management*, Method **998.01**, *Synthetic Pyrethroids*, was also declared Official Final Action. The topics *Determination of Residues of Triazines and their Chloro-Metabolites in Raw Agricultural Commodities* and *Dioxins by GC/MS* remain vacant.

General Topic Review

A Handbook of Residue Analytical Methods for Agrochemicals, Philip Lee (Ed.), has been published this year by Wiley. From its prospectus, this publication seems to address regulatory issues, and the required structure of analytical studies for regulatory purposes as much, or more, than it does analytical methodology, per se. This reference nicely addresses methods for newer pesticides, rather than once again addressing the usual older organophosphates and chlorinated hydrocarbons. Vol. 1 appears to loosely focus on chromatographic methods and Vol. 2 on immunological and other nonchromatic methods. An *Encyclopedia of Agrochemicals*, Jack Plimmer (Ed.), has also been published by Wiley this year. This reference looks from its prospectus to be an attempt at fairly comprehensive coverage of issues of general interest to pesticide chemists. Although there are chapters dedicated to CIPAC, HPLC, LC/MS, etc., it is unclear if specific analytical procedures are described within these chapters, and discussion of analytical chemistry does not appear to be a primary purpose of the encyclopedia.

Vol. 1000 (2 issues) of the *Journal of Chromatography, Part A*, is dedicated to excellent reviews of chromatographic methods under the title: "A Century of Chromatography." The reviews are not historical, but cover a host of current chromatographic and sample preparation techniques. Although the

reviews are not specifically aimed at pesticide analyses, pesticide residue chemists will find a lot of useful information in these reviews.

The 2nd Ed. of A. Schecter's *Dioxins and Health* was also published this year, but covers only toxicology, not analytical procedures. The National Academy of Sciences (NAS) is in the process of publishing *Dioxins and Dioxin-Like Compounds in the Food Supply: Strategies to Decrease Exposure*. This report is not in print yet, but a prepublication version is currently (July 2003) available at the NAS Web site. This NAS report recommends that additional analyses for dioxins should be performed to fill exposure data gaps, and that cheaper, faster methods of dioxin analysis need to be developed. While on the topic of dioxins and other persistent organic pollutants (POPs) it is also worth noting that Domingo et al. (1) reported on "Polychlorinated Naphthalenes in Food: Estimated Dietary Intake by the Population of Catalonia, Spain." Domingo et al. found that, while chlorinated naphthalenes are not present in food in large enough quantities to present a hazard on their own, they may contribute to the overall load of POPs. There is apparently a higher association of these chemicals with cereals and vegetable oils than with animal products. In the same line, Rayne (2) reported in "Rapidly Increasing Polybrominated Diphenyl Ether Concentrations in the Columbia River System from 1992 to 2002" that polybrominated Diphenyl Ether Concentrations may be increasing to above PCB concentrations in whitefish taken from the Columbia River system.

EPA's Analytical Chemistry Laboratory (ACL) branch has developed an index of all of the pesticide residue methods that have been tested at ACL. This index is posted on the EPA Web site at <http://www.epa.gov/oppbead1/methods/ram12b.htm>. This site contains all food and feed methods verified in ACL since 1980, including some methods not listed in PAM. In addition, ACL has now posted TIF images of the complete methods for about 75% of the listings. Individual methods not yet posted as TIF images may be requested from ACL. This site must now undoubtedly be considered the premier online resource for analytical residue methods for individual pesticides in foods.

With the advent of LC/MS/MS and other chromatography/MS^x techniques for pesticide residue analysis have come 2 important issues: (1) criteria for regulatory confirmation with these techniques, and (2) questions about matrix effects using some of these techniques. The U.S. Food and Drug Administration/Center for Veterinary Medicine (FDA/CVM) has just finalized their guidelines confirmation of animal drug residues by these techniques. The final guidelines are posted on the FDA Web site at <http://www.fda.gov/cvm/guidance/dguide118.PDF>. These guidelines, of course, do not cover pesticides residues in foods, but they provide a good ap-

proach for confirming pesticide residues. The closest competing system of which this General Referee is aware is a point system for confirmations that was developed in Europe. This system has undoubtedly since been considerably updated, but the last version the General Referee has seen in print was "Trends in the Identification of Organic Residues and Contaminants: EC Regulations under Revision" by Andre et al. (3). Betham and other members of a committee (4) from the American Association of Mass Spectroscopy (ASMS) have also published some thoughts on the closely related issue of "Establishing the fitness for purpose of mass spectrometric methods," but this discussion is general, and does not contain a set of specific confirmation criteria.

On the issue of matrix effects, Hajslova et al. (5) have published a general review on "Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrixes." This review covers matrix effects in chromatography in general. A more specific article, "Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on LC/MS/MS," by Matuszewski et al. (6), covers the problem of matrix effects in LC/MS. Cuadros-Rodriguez et al. (7) have also published a "Correction function on biased results due to matrix effects—application to the routine analysis of pesticide residues." This paper attempts a mathematical approach to determining matrix effects in chromatographic results.

Several papers on multiresidue pesticide methodology have been published. Taylor et al. (8) published a "Multiresidue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry." This procedure describes an LC/MS/MS technique in which grapes, kiwi fruits, lemon, spinach, etc., are extracted, the extracts are simply filtered, and the LC and first stage of the MS are used to provide adequate cleanup for detection of representative daughter ions for each pesticide. Arrebola et al. (9) have published a "Determination of 81 multiclass pesticides in fresh foodstuffs by a single injection analysis using gas chromatography—chemical ionization and electron ionization tandem mass spectrometry." This method is an attempt to speed up the analysis by taking advantage of GC/MS/MS.

Kochman (10) has discussed "Fast, high sensitivity, multipesticide analysis of complex mixtures with supersonic gas chromatography—mass spectrometry." Supersonic—mass spectrometer interface provides the molecular ion in higher yield for each pesticide, complemented by lower background. These effects, in turn, enable such things as better sensitivity and/or full scan mass spectroscopy, better comparison to identification databases, and a potential for increased chromatographic speed. Obviously this is a technique with much promise for multiresidue pesticide analysis. [A discussion of the supersonic interface can also be found on Aviv Amirav's home page at [http://www.tau.ac.il/chemistry/amirav/.](http://www.tau.ac.il/chemistry/amirav/)] Lehotay (11), in "Application of Gas Chromatography in Food Analysis," and Mastovska (12), in "Practical Approaches to Fast Gas Chromatography—Mass spectrometry," discuss this and other recent techniques for performing

multiresidue pesticide (and other analyses) in food by GC/MS at very high speeds.

Poustka et al. (13) have discussed the "Application of supercritical fluid extraction in multiresidue pesticide analysis of plant matrixes" and Rosenblum (14) has made a "Comparison of five extraction methods for determination of incurred and added pesticides in dietary composites."

Many single analyte, or single class, pesticide methods have been published within the last year. Among them are a very large number of immunoassays for pesticides. It has not been possible to look at all of these procedures, so the following are a sampling of the methods that, for one reason or another, seemed more significant. Yague (15) published a rapid method for trace determination of organochlorine and polychlorinated biphenyls in yogurt. Song (16) published "Fast chromatography analysis of N-carbamates with cold on-column injection." Pogacnik (17) published "Detection of organophosphate and carbamate pesticides in vegetable samples by a photothermal biosensor". Schulze (18) published a "Design of acetylcholinesterases for biosensor application." Zhu (19) reported on a "Molecularly imprinted polymer for metsulfuron-methyl and its binding characteristics for sulfonylurea herbicides." Frenich (20) studied "Standardization of SPE signals in multicomponent analysis of three benzimidazole pesticides by spectrofluorometry." Nogueira (21) reported an LC/MS method for carbamates in "Considerations on ultra trace analysis of carbamates in water samples." Little (22) reported the "On-line preconcentration of atrazine by antibody immobilization capillary electrophoresis." Rosales-Conrado (23) reported a "Determination of chlorophenoxy acid herbicides and their esters in soil by capillary liquid chromatography with ultraviolet detection, using large volume injection and temperature gradient." Finally, Pico (24) surveyed use of capillary electrophoresis for a variety of single analyte or single class pesticide methods in "Capillary electrophoresis for the determination of pesticide residues."

Note that any opinions expressed here are those of the General Referee and not those of the EPA.

Selected Study Director Topics

Chlorinated Dioxins

Topic Advisor Douglas Hayward, U.S. Food and Drug Administration (FDA), HFS 336, 200 C St, SW, Washington, DC 20204, Tel: +1-202-205-4406, Fax: +1-202-205-4422, E-mail: douglas.hayward@cfsan.fda.gov. Hayward reports progress during half of this year has been severely limited due to the relocation of the FDA Center for Food Safety and Applied Nutrition to its new location at 5100 Paint Branch Pkwy, College Park, MD 20740. Laboratory space was limited at first, but more space was found over the winter. The dioxin method originally designed for the older Saturn 3 ion trap has already been updated last year for the new Saturn 2000 and now includes the dioxin-like PCBs 77, 126, and 169. A document describing these new procedures is making its way to the

field laboratories this spring for testing with both tandem mass spectrometry (MS/MS) and high resolution mass spectrometry (HRMS).

Sample preparation procedures covering fish, dairy products, and the total diet foods are modified where necessary to accommodate the PCBs. Testing for actual food samples with the new procedures has been moving forward during the winter and spring of 2002/03. In addition, high volume injection was tested for dioxins this spring and appears to work well for the Saturn 2000 instruments without any modification of the GC. High volume injection will permit a 60–80% reduction of the test portion size with a corresponding reduction in solvent and reagent use during extraction and cleanup. High volume injection is the first step in a planned effort to automate the analysis of dioxins, PCBs, and PBDEs. The greatest difficulty with high volume injection is obtaining a sufficiently low PCB blank so that limits of quantitation (LOQs) will not be affected by the scaling down of sample size.

The conditions for a separate MS/MS acquisition targeted for 22 other PCB congeners isomer specifically from a different fraction of the same food sample test portion as use for the dioxins was completed this past winter. The 22 PCBs include some dioxin-like mono-ortho-chlorine substituted PCB congeners and others that are major components of environmental samples. They are as follows: PCBs 28, 37, 44, 49, 52, 66, 74, 95, 99, 101, 105, 110, 114, 118, 137, 138, 153, 156, 157, 170, 180, and 187. The quantification of all PCB congeners will be done using an internal standard method with the following $^{13}\text{C}_{12}$ uniformly labeled standards: PCB 28, 52, 70, 77, 101, 114, 118, 126, 138, 153, 156, 157, 169, 170, and 180. Both these MS/MS procedures are being tested this summer with a number of dairy and fish matrixes. Freeze drying of fish for dioxin and PCB analysis is also being tested as a further aid to an automated procedure and to improve the LOQs for the dioxins.

2000.05 Determination of Glyphosate and (Aminomethyl) Phosphonic Acid in Crops by Capillary Gas Chromatography with Selective Detection

Study Director Phil Alferness, Biomarin Pharmaceuticals, Inc., Ste 210, Analytical Biochemistry Department, 371 Bel Marin Keys Blvd, Novato, CA, tel: +1-415-506-6121, Fax: +1-415-382-0113, E-mail: palferness@biomarinpharm.com. This method was made Final Action this year (*Inside Laboratory Management*, May/June 2003). Alferness reports that he is now distanced from this method and also has attempted, but has been unable, to locate someone who can continue as Method Advisor. Therefore, this topic should be discontinued.

Determination of Residues of Triazines and Their Chloro-Metabolites in Raw Agricultural Commodities

This topic is currently vacant. Continue topic for 1 more year.

Dioxins by GC/MS

This topic is currently vacant. Because there is no current interest in this particular topic and because it largely overlaps

with the other dioxin topic, which is active, the General Referee recommends that this topic be discontinued.

2002.03 Pesticides in Nonfatty Foods Using SFE and GC/MS

Steven J. Lehotay, U.S. Department of Agriculture, Agricultural Research Service, Regional Research Center, Food Safety Research Unit, 600 East Mermaid Ln, Wyndmoor, PA 19038, Tel: +1-215-233-6433, Fax: +1-215-233-6642, E-mail: slehotay@arserrc.gov. Study Director Lehotay reports that this collaborative study became First Action Official Method **2002.03** of AOAC INTERNATIONAL, and the collaborative study manuscript was published in *J. AOAC Int.* **85**, 1148–1166 (2002; 25). The Study Director has received several reprint requests for the publication. SFE instrument manufacturers report a greater interest in SFE, especially in Asia, since the method became an AOAC Official Method.

Pesticides in Foods Using Acetonitrile Extraction and Partitioning with Magnesium Sulfate

Steven J. Lehotay, U.S. Department of Agriculture, Agricultural Research Service, Regional Research Center, Food Safety Research Unit, 600 East Mermaid Ln, Wyndmoor, PA 19038, Tel: +1-215-233-6433, Fax: +1-215-233-6642, E-mail: slehotay@arserrc.gov. Topic Advisor Lehotay reports that a fast and easy method was developed for the multiclass, multiresidue analysis of pesticides in fruits and vegetables that uses acetonitrile for extraction, a combination of magnesium sulfate and sodium chloride for salting out the water, and dispersive solid-phase extraction for cleanup. See ref. 26 for the comprehensive publication that introduces this promising new method. To provide a short name for the method, it has been dubbed the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach to pesticide residue analysis. In consultation with the General Referee and Committee on Residues and Related Topics, Lehotay has become a Methods Investigator and proposes that the method be evaluated in a collaborative study. In the coming months, he will submit a protocol for review by the GR and committee. More than 10 laboratories have already been identified in several countries to conduct the interlaboratory trial.

The original method is very streamlined, consisting of few, simple steps as follows: (1) weigh 10 g well-comminuted sample into a Teflon centrifuge tube or bottle and add 10 mL acetonitrile (MeCN); shake or mix in a Vortex mixer well for 1 min; (2) add 4 g anhydrous MgSO_4 + 1 g NaCl (+ an internal standard) and shake/mix in a Vortex mixer for 1 min; (3) centrifuge at 1500 rcf for 1 min; (4) transfer 1 mL MeCN extract to a 2 mL centrifuge tube containing 150 mg anhydrous MgSO_4 + 25 mg primary secondary amine sorbent (PSA); mix for 30 s; and (5) remove 0.5 mL extract and inject in gas chromatography/mass spectrometry (GC/MS). The method can be easily scaled to larger or smaller sample sizes by keeping the sample:reagent ratios the same. For detection, the method is also compatible with a variety of detection systems except for the nitrogen–phosphorus and electrolytic conductivity detectors in GC due to the MeCN solvent.

In the original study, approximately 25 diverse pesticides for GC/MS were evaluated, and since then, more than 200 other pesticides in several matrixes and different detection systems have also been tested among different laboratories. The experiments have demonstrated recoveries to be 90–110% for a wide polarity range of pesticides, including *n*-methylcarbamates, conazoles, pyrethroids, organophosphates, organochlorines, triazines, and several other classes. Many important pesticides, such as dichlorvos, imazalil, thiabendazole, methamidophos, acephate, and aldicarb (including sulfone and sulfoxide), that are difficult by traditional methods, give consistent 80–100% recoveries in the QuEChERS method. The PSA will remove pesticides that contain carboxylic acids (such as daminozide), and the very hydrophilic pesticides (such as asulam) do not completely partition into the MeCN extract. Dichlofluanid, tolyfluanid, captan, folpet, captafol, chlorothalonil, and dicofol tend to degrade in the MeCN extract, but this can be reduced by keeping the pH low and using dark vials. These are problematic pesticides by any multiclass, multiresidue pesticide method. Further experiments will be conducted to fine tune the method to achieve the most efficient approach for rapid extraction of the widest practical range of pesticides and still achieve 10 ng/g LOQs for residues in foods with <15% lipid content.

Miniaturized Methods

Frank Schenk, FDA, Southeastern Regional Research Laboratory, 60 Eighth St, NE, Atlanta, GA 30309, Tel: +1-404-253-1200, Fax: +1-404-253-1208, E-mail: fschenk@ora.fda.gov. Topic Advisor Schenk compared a recently published rapid multiresidue method for the determination of pesticide residues in produce (26) to 2 other published methods. Eleven fresh produce samples containing various incurred pesticide residues were analyzed using the new rapid multiresidue method, the published acetonitrile extraction method used by the Canadian Pest Management Regulatory Agency (27) and the acetone extraction (Luke) method used by the FDA (28). Incurred pesticide residues studied included azinphos methyl, carbaryl, dimethoate, endosulfans I and II, sulfate, omethoate, phosmet, as well as 3 samples containing ca 10, 60, and 700 ng/g methamidophos. Similar results were obtained for all the samples using all 3 methods.

998.01 Synthetic Pyrethroids

Method Advisor Guo-Fang Pang, Qinhuangdao Entry-Exit and Quarantine Bureau, No. 39 Haibin Rd, P.C. 066002, Qinhuangdao, People's Republic of China, Tel/Fax: +86/335-341-7119, E-mail: panggfciq@pang.com.cn. This method was adopted Final Action. Method Advisor Pang reports that there are 9 papers related to the study of the said topic, which have been published in *J. AOAC Int.* and *J. Chromatogr.*, and another 5 were entered into the posters on 109th and 112th AOAC Annual Meetings. The publication of these papers aroused great interest of international counterparts. Between 1995 and 2002, 112 scholars from 28 countries over the 5 continents solicited for the papers for a total of 159 times. At the same time, 190 scholars from 22 countries

cited the above-mentioned 9 papers 80 times in their 51 published papers on 32 journals based on the Scientific Citation Index (SCI). Therefore, the study gave Pang "tremendous joy and relief," and he wishes to extend sincere appreciation to his colleagues, especially Charles Parfitt of FDA, then GR, who gave personal instruction to the study. Pang has received no letters concerning problems encountered when using AOAC Method 998.01, since it was adopted First Action in 1998, but he wishes to continue as Method Advisor and would very much like to receive additional feedback on the utility and performance of the method.

Recommendations

(1) *Chlorinated Dioxins*: Topic Advisor Douglas Hayward. Continue topic.

(2) **2000.05** *Determination of Glyphosate and (Aminomethyl) Phosphonic Acid in Crops by Capillary Gas Chromatography with Selective Detection*: Study Director Phil Alferness. Method has been made Final Action. Discontinue topic.

(3) *Determination of Residues of Triazines and Their Chloro-Metabolites in Raw Agricultural Commodities*: Vacant. Continue topic.

(4) *Dioxins by GC/MS*: Vacant. Discontinue topic.

(5) **2002.03** *Pesticides in Nonfatty Foods Using SFE and GC/MS*: Method Advisor Steven J. Lehotay. Continue topic.

(6) *Pesticides in Foods Using Acetonitrile Extraction and Partitioning with Magnesium Sulfate*: Method Advisor Steven J. Lehotay. Initiate topic.

(7) *Miniaturized Methods*: Topic Advisor Frank Schenk. Continue topic.

(8) **998.01** *Synthetic Pyrethroids*: Method Advisor Guo-Fang Pang. Method has been made Final Action. Continue topic.

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