Report on Chlorinated Pesticides

By JERRY A. BURKE (Division of Chemical Technology, Food and Drug Administration, Washington, D.C. 20204)

Seven Associate Referees have reported to the General Referee on the current status of their work. The topics of Chlordane, Extraction Procedures (Blending-Type), Thin Layer Chromatography (Multiple Detection), and Toxaphene are vacant.

The Associate Referee on Chlorobenzilate, Chloropropylate, and Acaroi reports that none of these acaricides are completely recovered through the Florisil column chromatographic cleanup of the AOAC multiresidue methodology (29.001). The Associate Referee intends to investigate other elution solvents and column chromatographic systems for cleanup and recovery. It is recommended that this work be continued with the objective of modifying the basic AOAC methodology to obtain adequate analysis for these compounds.

The Associate Referee on Heptachlor and Heptachlor Epoxide has resigned because of other pressing commitments. No work has been done. It is recommended that studies be initiated to evaluate procedures for forming chemical derivatives suitable for residue identification, with the objective of adaptation to AOAC multiresidue methodology (29.001).

The Associate Referee on Kelthane has not been able to initiate the recommended collaborative study. It is recommended that the method for dicofol (Kelthane) (1), which utilizes a colorimetric determination, be studied collaboratively; the study should be completed prior to the 1972 meeting.

The Associate Referee on Multiple Pesticide Residues in Soils, due to other obligations, has done no work beyond that of last year. It is recommended that this investigation be continued to develop or adapt an efficient extraction procedure for use with AOAC multiresidue cleanup and determinative methodology.

The Associate Referee on Meats and Fatty Foods, W. M. Rogers, reported at the 1971 AOAC meeting on a procedure for the isolation of chlorinated insecticides from large samples of fats and oils. The increase in sample size achieved by this procedure may lead to improved determination of low level residues in fatty foods. The Referee concurs with the Associate Referee's recommendation that the procedure be exposed to practical application prior to decision on further development or collaborative study.

At the 1971 AOAC meeting, the Associate Referee on Perthane, R. T. Krause, reported a method for the quantitative determination of Perthane residues in fruits and vegetables. The method utilizes a gas chromatographic determination of the olefin which is formed by treatment of Perthane with alkali. The report includes information on the effect of the alkaline treatment on a large number of pesticide chemicals. The Referee concurs with the Associate Referee's recommendation for collaborative study of the method for Perthane, including the use of thin layer chromatography for confirmation of identity.

The Associate Referee on Tetradifon, Endosulfan, and Tetrasul was not able to continue his investigations during the past year. The General Referee recommends collaborative study of the Florisil column chromatographic procedure reported at the 1971 AOAC meeting (2), in conjunction with acetonitrile extraction (29.009) and GLC determination (29.017) of AOAC multiresidue methodology, for these pesticides and endosulfan sulfate in fruits and vegetables.

Associate Referees should be appointed to the vacant topics: (*1*) Chlordane: to continue studies on the nature and determination of residues; (*2*) Extraction Procedures (Blending-Type): to initiate studies to replace ineffective extraction procedures in current AOAC methods with more reliable blending-type extraction (initial work should be with methods for captan (29.061) and parathion (29.039)); (*3*) Thin Layer Chromatography (Multiple Residues): to study further application of this technique to residue identification; (*4*) Toxaphene: to initiate collaborative study of the procedure (3) for DDT removal followed by GLC and colorimetric determination of toxaphene.

Four recommendations are made concerning AOAC multiresidue methodology:

(*1*) That the first action method for extraction of certain organochlorine pesticides from dry products (29.009(e)–29.014(b)) be made official final action for aldrin, BHC, *p,p'*-DDE, *p,p'*-DDT, endrin, heptachlor, lindane, methoxychlor, and *p,p'*-TDE in barley, corn meal, hay, oats, popcorn, and wheat.

(*2*) That the first action method for certain organochlorine pesticides in fish (29.010(e)) be made final action for BHC, *p,p'*-DDE, *p,p'*-DDT, dieldrin, heptachlor epoxide, and *p,p'*-TDE.

This report of the General Referee was presented at the 85th Annual Meeting of the AOAC, Oct. 11–14, 1971, at Washington, D.C.

Section numbers refer to *Official Methods of Analysis*, 11th Ed., 1970.

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(3) That the AOAC multiresidue method for certain organochlorine pesticides in fruits and vegetables (29.009) be collaboratively studied for determination of dieldrin, heptachlor epoxide, *o,p'*-DDT, and mirex.

(4) That the above collaborative study include validation of the widely used gas chromatographic column (4).

Multiresidue analytical methodology for organochlorine pesticides has reached the point where reasonably sound analyses can be made for a large number of chemicals in a large number of sample types. Two general limitations exist: (1) Every important pesticide chemical is not recovered through the same procedure, and (2) not all sample types can be handled in straightforward fashion. Obviously, for a particular analytical scheme to reach its fullest potential, orderly development must continue in order to improve upon the basic analytical methodology.

This is the approach we have taken for the multiresidue methodology contained in *Official Methods of Analysis*. For example, last year the procedures for extraction of organochlorine residues from fish and low moisture samples were added into the basic methodology. One recommendation this year is to extend the methodology for fruits and vegetables, through collaborative study, to additional important chemicals. Follow-through on the recommendation on endosulfan, tetradifon, and tetrasul should permit inclusion of these chemicals in established AOAC methodology with a change in only one part of the method. Furthermore, this study would represent a beginning of validation of the new and improved Florisil column cleanup.

In those cases where extraction efficiency may be questionable, e.g., root-absorbed residues, soils, or oilseeds, investigators are encouraged to work toward extraction procedures that fit into the already proven cleanup and determinative procedures.

Confirmation of residue identity is of utmost concern in multiresidue analyses and depends as much on the analyst's interpretations and judgment as on analytical procedures. Every residue analytical chemist must be concerned with the validity of his findings and perform the necessary work to assure that data are valid. In this area investigators are encouraged to develop confirmatory tests which are readily applicable to the extracts prepared for multiresidue analysis. This has been done by R. T. Krause, the Associate Referee for Perthane, whose presentation at the 1971 AOAC meeting not only described a method for this compound, but dealt with the effect of alkaline hydrolysis on many chemicals. This information should be most useful in confirming residue identity.

Polychlorinated biphenyls (PCB) have been and are now of much interest to the residue analyst. Current interest is primarily in detection and measurement of residues rather than in the interference potential of PCB in determination of organochlorine pesticides. Some means of dealing with the latter problem are available with such procedures as silicic acid column chromatography for separating some PCB from certain organochlorine pesticides (5), oxidation of DDT to permit separation from PCB by thin layer chromatography (6), and treatment with alkali to effect changes in some pesticides (Krause, 1971 AOAC meeting). In all probability, refinements of these procedures and development of additional ones will improve the ability to cope with PCB-pesticide residue mixtures. Recent study of the silicic acid column chromatographic procedure has shown that a complete separation of all components of lower chlorinated Aroclors (e.g., Aroclors 1248 and lower) from DDT and analogs is not achieved (7). Some of the lower chlorinated biphenyls are not completely eluted from the column with the 250 ml petroleum ether required to separate Aroclor 1254 from *p,p'*-DDE. Experience with this procedure has also shown the need, with some lots of silicic acid, to vary the water content slightly upward or downward from 3% to achieve optimum separation of Aroclor 1254 from *p,p'*-DDE. The current improved state of awareness of the residue analyst to the analytical characteristics of PCB is very important in regard to avoidance of PCB interference in determination of organochlorine pesticide residues.

Quantitation of PCB residues is a complex problem. There is no assurance that the residue will be the same composition as the commercial PCB (Aroclor) from which it derived. The majority of PCB residues found in wildlife and environmental samples, e.g., fish, birds, and sediments, have been reported to resemble Aroclor 1254 or 1260 in gas chromatographic patterns. Accidental spills or leaks have resulted in instances of direct contamination with particular Aroclors. However, there may be changes in the PCB mixture due to weathering or metabolism, and there is a possibility of encountering residues resulting from more than one Aroclor.

Analysis for residues of PCB can be performed with the same extraction, cleanup, and GLC and TLC detection procedures as for DDT. However, the multicomponent nature of PCB and the industrial use of different commercial PCB mixtures complicate the analysis. Response of the widely used electron capture GLC detector is not directly proportional to chlorine content of the chlorobiphenyl molecule (8). With the electron capture GLC parameters employed in the General Referee's laboratory, the area response to a given weight of Aroclor 1254 (54% chlorine) is approximately 2 times the response to the same weight of Aroclor 1248 (48% chlorine).

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The difference in response is even greater with higher or lower Aroclors. Because of this, the choice of an Aroclor as a quantitation reference can significantly affect the analytical result. For this reason extreme care and good judgment are imperative when the electron capture detector is used for quantitation of PCB residues. Use of the halogen-specific microcoulometric or electrolytic conductivity detector should reduce the difference between measurements based on different Aroclors as quantitation references. However, the lower sensitivity of these detectors (greater quantity required for detection), their lack of availability in many laboratories, and operational difficulties preclude restricting quantitation of PCB residues to their use.

All components of the PCB mixture may be recovered to the same degree through the various operations of sample extraction and cleanup. Investigations in the General Referee's laboratory have shown that recovery through the petroleum ether to acetonitrile partitioning (29.010(e)) decreases with increasing chlorine content of the PCB. For example, recovery of Aroclor 1254 was 87% while recovery of Aroclor 1242 was 97%. Recoveries of individual components of Aroclors also tended to decrease with increasing chlorine content of the particular component. Different behavior of Arochlors in silicic acid column chromatography was discussed earlier.

An interlaboratory study conducted by the General Referee's laboratory showed the average level of Aroclor 1254 reported by 14 laboratories to be about 75% of the amount added to the study samples. Rainbow trout was fortified with either 2 or 0 ppm Aroclor 1254 and 3, 4, and 1 ppm p,p'-DDE, p,p'-DDT, and p,p'-TDE, respectively. Section 29.010(e) was used for analysis, together with the Armour-Burke procedure for separation of the PCB from the organochlorine pesticides prior to electron capture GLC determination. Levels of p,p'-DDT, p,p'-DDE, and p,p'-TDE reported averaged 89, 85, and 89%, respectively, of the quantities added. This work indicates that the PCB potential for interference with determination of DDT and analogs can be satisfactorily handled, but that PCB residue values, in general, may tend to be slightly lower than the actual amounts. It must be kept in mind that in this case the PCB determined was an added Aroclor unchanged in composition and involved no question in choice of the Aroclor quantitation reference.

When the PCB residue is altered in some fashion from the commercial PCB (Aroclor) or when the residue appears to be derived from more than one Aroclor, then the best means of quantitation become less certain. However, adherence to a particular procedure should enhance interlaboratory agreement. Experience in the General Referee's laboratory, and in other laboratories with which we have communicated, indicates that the following is currently the most desirable and practical approach to the quantitative estimation of PCB residues: "Perform extraction and cleanup as for DDT, preferably using 29.001. Examine extract by GLC (electron capture or halogen-specific microcoulometric or electrolytic conductivity detection), operating the GLC system at standardized conditions. Carefully evaluate chromatographic record from sample. Compare retention times of peaks from sample to retention time data for organochlorine pesticides and Aroclors. Evaluate relative component (peak) composition in chromatogram from suspected PCB residue vs. relative component composition of Aroclors. If interfering organochlorine pesticides are present, use appropriate means, such as separation and/or chemical treatment discussed above, to compensate for or to remove the pesticides. Choose for quantitation reference the Aroclor or Aroclors with the GLC pattern most similar to that of sample. Estimate PCB residue quantity by comparing the total peak area for sample to total peak area for known amount of the appropriate Aroclor(s). If the residue clearly consists of components of more than one Aroclor, when possible, quantitate and report the residues separately, using the appropriate Aroclor reference for the respective portions of the GLC curve. Recoveries of different Aroclors through the complete analytical procedure should be known. Confirm residue identity, using halogen-specific microcoulometric or electrolytic conductivity GLC to show that characteristic GLC pattern is from halogenated compounds, alkali reflux to demonstrate stability of the residue, and/or a TLC procedure that is suitably specific for PCB."

It does not appear possible at this time to prescribe a definite analytical scheme which can completely cope with all the variables in quantitative determination of PCB residues. However, residues of PCB are a fact of life. These residues are readily detectable and as a practical matter require quantitative estimation. Until a definite quantitation procedure is developed, laboratories are encouraged to exercise good judgment in their measurement of PCB residues and to describe explicitly the analytical procedures employed when reporting residues of PCB. If the complexities of PCB residue determination are recognized, limitations of this analysis are understood, and analyses are conducted only by experienced residue analytical chemists, then misleading residue data can be avoided.

Recommendations

It is recommended—

(1) That the general method for pesticide residues, 29.001–29.027 and revisions in (1971) *JAOAC* 54, 470, be adopted official final action for (A) aldrin, BHC, p,p'-DDE, p,p'-DDT, endrin, heptachlor,

lindane, methoxychlor, mirex, nonachlor, oxychlordane, toxaphene, and trans-nonachlor, 29.014(b); aldrin, dieldrin, heptachlor epoxide, and mirex, 29.010(e).

(2) That Appendix VI, Recommendations for the following topics: Preparation of Aroclor Solutions (Blending-Type Solvents), Preparation of Aroclor Solutions (Thin Layer Chromatography), and Toxicity.

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lindane, methoxychlor, and *p,p'*-TDE in barley, corn meal, hay, oats, popcorn, and wheat, 29.009(e)–29.014(b); and (B) BHC, *p,p'*-DDE, *p,p'*-DDT, dieldrin, heptachlor epoxide, and *p,p'*-TDE in fish, 29.010(e).

(2) That Associate Referees be appointed on the following topics: Chlordane, Extraction Procedures (Blending-Type), Heptachlor and Heptachlor Epoxide, Thin Layer Chromatography (Multiple Residues), and Toxaphene.

(3) That study be continued on all other topics.

The recommendations of the General Referee were approved by Subcommittee E and adopted by the Association; see the report of the Subcommittee for detailed recommendations (p. 324) and "Changes in Methods."

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Report on Fungicides, Herbicides, and Plant Growth Regulators

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Despite adverse publicity of pesticides, new compounds are still being developed. This should be encouraged, providing that these new compounds are more selective in action and less persistent in nature. Pesticides still remain the most widely used weapon in the farmers' fight against pests.

New Herbicides Reported

Bromofenoxin (C-9122), 3,5-dibromo-4-hydroxybenzaldoxime - 2',4' - dinitrophenyl ether (CIBA Corp.)

Chlornidine (Torpedo), N,N-di-(2-chloroethyl)-4-methyl-2,6-dinitroaniline (Ansul Co.)

Dicuran (Chlortoluron), N-(3-chloro-4-methylphenyl)-N',N'-dimethylurea (CIBA Corp.)

Diethamine (USB 3584), N^3,N^3-diethyl-2,4-dinitro - 6 - trifluoromethyl - 1,3 - phenylenediamine (U.S. Borax Research Corp.)

3-Isopropyl-1*H*-2,1,3-benzothiadiazin-(4)3*H*-one 2,2-dioxide (BASF Wyandotte Corp.)

SAN 9789, 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro - *m* - tolyl) - 3(2*H*) - pyridazinone (Sandoz-Wander, Inc.)

Recent Analytical Procedures

Fungicides

Parinol (α,α - bis(p - Chlorophenyl) - 3 - pyridine - methanol).—Methods have been developed for both formulations and residues (1). The macro methods included nonaqueous titration, IR, UV, colorimetry, and GLC. For residue determination, a sample was blended with acetone, filtered, and then partitioned into hexane. The residue was extracted into 0.5*N* HCl and, after neutralizing, extracted with chloroform. A 1.5% OV-17 column was used for ECGLC (electron capture gas-liquid chromatography). TLC can also be used for residue monitoring. After extraction and cleanup, the residue was spotted on silica gel and developed with ether-hexane-methanol (90 + 18 + 2). Spots were revealed with 10% H_2SO_4 in ether. Sensitivity of the methods is 0.1 ppm.

Vitavax.—A gas chromatographic method, utilizing the microcoulometric nitrogen detector, has been developed for vitavax and its sulfoxide in cereal grains (2). Samples were extracted with methanol in a Soxhlet apparatus. Residues were extracted into chloroform and then hydrolyzed with base to form the aniline, which was isolated for GLC determination. Crop blanks of about 0.1 ppm will limit the usefulness of this method.

Herbicides and Plant Growth Regulators

Arsenicals.—In the colorimetric determination of arsenic, a paper chromatographic separation procedure was introduced to separate cacodylic acid (hydroxydimethylarsine oxide), MSMA (monosodium methanearsonate), sodium arsenate, and sodium arsenite (3). Samples were extracted with water, filtered, and evaporated to dryness. A 50% methanol solution was added to the residue and the mixture

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