Journal
of the
Association of Official Analytical Chemists

Editorial Board
DANIEL BANES, *Chairman*

EDITORIAL COMMITTEE

WILLIAM HORWITZ A. P. MATHERS
PETER CHICHILO F. W. QUACKENBUSH
I. HOFFMAN C. O. WILLITS
HELEN L. REYNOLDS

STAFF

DANIEL BANES, *Editor-in-Chief*
HELEN L. REYNOLDS, *Editor*
[illegible] VÁCH, *Managing Editor*
[illegible] CK, *Assistant Editor*
MARIE L. TALLEY, *Assistant Editor*
W. M. HOFFMAN, *Book Review Editor*

VOL. 50 OCTOBER 1967 NO. 5

CONTENTS

PAGE

Golden Jubilee Papers

The Continuing Problem of Sampling. By F. W. Quackenbush and R. C. Rund.. 997
The Role of Statistics in Regulatory Work. By W. J. Youden 1007
Analytical Methods and the Preclearance Food Protection Amendments to the FD&C Act. By L. L. Ramsey 1013
Magic Words—Automated Methods of Analysis. By H. P. Eiduson and W. M. Hoffman 1021
Developments in Adulteration of Food and Its Detection. By William Horwitz and Helen L. Reynolds 1024
Drug Adulterations—Past, Present, and Future. By Daniel Banes 1032
Advanced Instrumentation in Forensic Examinations. By M. J. Pro and C. M. Hoffman 1041
Is Chemistry Going Out of Analytical Chemistry? By Clifton E. Meloan 1044
The Impact of Instrumentation Upon the Teaching of Analytical Chemistry. By Stanley Bruckenstein 1046
Screening Methods in Regulatory Analyses. By R. E. Duggan 1052
Comments on Screening and Analysis of Multiple Pesticide Residues. By Milton S. Schechter and Melvin E. Getz 1056
Journal of the AOAC—The First 50 Volumes. By Helen L. Reynolds 1062

PUBLISHED BIMONTHLY BY
THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, INC.
Printed and published, third floor, 414 Water Street, Baltimore, Maryland 21202
Editorial and Advertising Office: Box 540, Benjamin Franklin Station, Washington, D.C. 20044
Phone: Area Code 202 962-4809

Copyright, 1967, by the Association of Official Analytical Chemists, Inc.

Entered as second class matter at the post office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in the Act of February 28, 1915, embodied in paragraph 4, section 412 P. L. & R. authorized March 25, 1931. Issued six times a year in February, April, June, August, October, and December. Printed in U.S.A.

EXHIBIT
WBP M-17

MON-MT-008563

LEXOLDMON000071
WATER_PCB-00035538

PAGE

Pesticide Residues

IUPAC Commission on the Development, Improvement, and Standardization of Methods of Pesticide Residue Analysis. By H. Egan, *Secretary to the Commission* 1067

IUPAC Commission on Terminal Residues. By H. Egan, *Secretary to the Commission* 1071

Direct Fluorescent Detection of Organothiophosphorus Pesticides and Some of Their Sulfur-Containing Breakdown Products After Thin Layer Chromatography. By Mohamed Tawfik H. Ragab 1088

Determination of 2,4-D and Its Butoxyethanol Ester in Oysters by Gas Chromatography. By J. R. Duffy and Patricia Shelfoon 1098

Improved Method for the Determination of Ethylenebisdithiocarbamate Residues in Plants, Fruits, and Vegetables. By Charles F. Gordon, Richard J. Schuckert, and William E. Bornak 1102

Persistence of Sevin Residues on Some Vegetable Crops After Various Harvest Times. By Makarem Ahmed Elessawi and Abdel Rahman El-Refai 1109

Oils, Fats, and Waxes

Correlation of Fatty Acid Structure with Preferential Order of Urea Complex Formation. By John L. Iverson and R. W. Weik 1111

Programmed Temperature Gas Chromatographic Technique for Detecting Trace Amounts of Fatty Acids. By John L. Iverson 1118

Preservatives and Artificial Sweeteners

Rapid Determination of Sorbic Acid in Orange Juice. By Kermit M. Floyd 1123

Preservatives: A Review of Methods of Analysis. By Pieter L. Schuller and Eduard Veen 1127

Entomology

Fly Identification by the Morphology of the Head and Head Appendages. By N. Aubrey Carson and Edmund F. Martinez 1146

Fruits and Fruit Products

Determination of Bitterness of Olives. By S. Cohen, A. Lifshitz, and Z. Samish.. 1194

Instrumental Techniques

Detection of Solvent by Infrared Spectrophotometry. By George Schwartzman, Daniel Sullivan, and Evelyn Sarnoff 1196

Color Additives

Determination of 1-Phenylazo-2-naphthol in D&C Red No. 17. By John C. Molitor 1198

Determination of 4(4-Chloro-2-sulfo-5-tolylazo)-1-naphthol in D&C Red Nos. 8 and 9. By Charles Stein 1199

Mycotoxins

Note on a Water-Based Aflatoxin Standard. By Robert Peterson and A. Ciegler.. 1201

Book Reviews 1203

MON-MT-008564

LEXOLDMON000072
WATER_PCB-00035539

PESTICIDE RESIDUES

IUPAC Commission on the Development, Improvement, and Standardization of Methods of Pesticide Residue Analysis

By H. EGAN, *Secretary to the Commission* (Laboratory of the Government Chemist, Cornwall House, Stamford St., London, S.E.1, England)

The first meetings of the above Commission of the Pesticides Section of the Applied Chemistry Division of IUPAC were held in Geneva in November 1966 under the chairmanship of Dr. R. A. E. Galley. The following account of the proceedings is based on the minutes of the meeting and on the appendixes presented by the members and associate members of the Commission as indicated.

1. Fumigant Residue Analysis

The Commission agreed that it was desirable to develop rapid, sensitive multidetection systems of analysis for residues of fumigants such as methyl bromide, ethylene dibromide, ethylene dichloride, ethylene oxide, acrylonitrile, carbon tetrachloride, carbon disulfide, and phosphine. A study of the application of gas-liquid chromatographic methods was thought to be worthwhile. A need for simple and reliable "on site" test equipment was also discussed and attention was drawn to limitations in the use of commercial indicator tubes for measurement of residues in treated produce.

2. Diphenyl Residue Analysis

The Commission discussed progress in the study of methods of sampling and analysis of citrus fruit for residues of diphenyl. A preliminary report of the Netherlands Central Institute for Nutrition and Food Research T.N.O. indicated that, of the two methods studied, the gas chromatographic method was the faster and more accurate; however, the thin layer chromatographic method followed by ultraviolet spectrophotometry was also suitable. Work on sampling boxed fruit had been completed and investigation of sampling procedures for shiploads and other consignments was in progress. Both methods of residue analysis appeared to the Commission to be acceptable.

3. Organomercury Fungicide Residue Analysis

Regulatory methods for organomercury residue analysis were discussed; it was indicated that while radioactivation facilities for this were adequate in Scandinavia, gas-liquid chromatography offered considerable promise both for high sensitivity and high specificity. The neutron activation methods were probably the best where facilities were available but, like the newer cold vapor atomic absorption techniques which were likely to be cheaper and just as sensitive, did not distinguish the form in which the mercury was bound. In the United States, the Food and Drug Administration made a direct comparison between cold vapor atomic absorption and neutron activation techniques; the Association of Official Analytical Chemists had recently appointed a new referee for mercury residue analysis.

4. Organochlorine Pesticide Residue Analysis

(a) Evaluation—by J. William Cook (Division of Food Chemistry, Food and Drug Administration, Washington, D.C. 20204)

Much progress has been made in the development and refinement of methods of analysis by which any or all of a large number of pesticide residue chemicals can be detected and measured in one general operation. They have come to be known as "multidetection" methods of analysis. The following discussion includes DDT, γ-BHC, aldrin, dieldrin, heptachlor, endrin, chlordane, endosulfan, and many other chemicals. There are a few hundred pesticide chemicals in use; some of these consist of more than

MON-MT-008565

LEXOLDMON000073
WATER_PCB-00035540

one compound and some convert to a series of alteration products which may appear in residue analysis. Therefore, there is a large number of chemicals which can show up in a *multidetection* method of analysis. Whether or not they are significant as residues, their presence must be recognized in order not to confuse them with significant pesticide chemicals. Ideally, a general method of analysis which would measure and identify all of the significant residues in all food products with a minimum of analytical effort and time would be desirable. This is not possible at the present time but some important achievements in that direction have been obtained to date.

The developments so far have made it possible to extract, clean up, measure quantitatively, and give a high degree of certainty to the identity of the compounds for up to 100 pesticide residue chemicals obtained from a great variety of food crops and products all in one general operation. This has been done with either extracts from individual products or composites of a large number of products, e.g., "total diet" or "market basket" samples. The development of such procedures has required a tremendous amount of effort. At each point along the way choices had to be made. As a consequence of these choices, adjustments in the total procedure have also had to be made. The choices may not necessarily have been the best ones, but as the total procedure developed, the more difficult it became to undertake any substitutions. Many of the steps add to the certainty of the identity of the chemicals as well as to the quantitative measurement. The extraction and the cleanup procedures must yield quantitative recoveries of the chemicals. The gas chromatographic systems—that is, combinations of column packing, liquid phase, temperature of operation, kind of detector, and operating parameters—are all highly important when one is attempting to obtain quantitative response from a number of chemicals. Conditions which may be satisfactory for quantitative results on an individual chemical or a small number of chemicals may not yield adequate quantitation for all of many chemicals.

The collaborative study of multidetection methods is not as straightforward as for "wet" methods of analysis. The relatively simple procedure that has been used by AOAC and other groups for design and execution of collaborative studies is generally not applicable to these multidetection procedures. It has been the experience of FDA and some other groups that if the details of the procedure are not described exactly and if they are not followed closely, great variations in the analytical results are obtained. At the last AOAC meeting, a special conference was held to discuss the problem involved in collaborative studies on these methods. It was decided that a special referee for collaborative studies would be appointed; he would work with the specialist in methodology for the organochlorines, organophosphates, herbicides, etc., to come up with total procedures which are ready for collaborative study if possible, and then describe those procedures, obtain collaborators, submit samples, receive and tabulate data, etc. It was proposed and accepted that if members of IUPAC were available to participate in this collaborative effort they would be welcomed by AOAC. It is anticipated that this program will become effective early in 1967. It will probably include extraction, cleanup, thin layer chromatography, and gas chromatography.

Gas chromatography will involve the use of an electron capture detector and a thermionic detector on a split stream or in tandem so that a separate but simultaneous readout for both organochlorines and organophosphates will be made. The simultaneous readout from the two detectors adds to the certainty of the identity of the compounds. For instance, parathion gives a large response on the thermionic detector due to the phosphorus and a much smaller response on the EC detector due to the NO_2 group. The presence and ratio of these two peaks along with retention time are quite characteristic. Likewise, trithion gives response by both detectors because of the phosphorus and the chlorine in the molecule. Again the retention time and ratio of the two responses enhance identification of the compounds being analyzed.

MON-MT-008566

LEXOLDMON000074
WATER_PCB-00035541

(b) Possible Interference by Chlorinated Biphenyls—by G. Widmark (Institute of Analytical Chemistry, University of Stockholm, Roslagsvagën 90, Stockholm 50, Sweden)

A large number of unknown but chlorine-containing compounds have been detected in addition to the ordinary pesticides in the analysis of samples, using both the electron capture and the microcoulometric detector. Mass spectra of some of these compounds obtained with a combined gas chromatograph-mass spectrometer (LKB-9000) indicated that most of the unknown compounds are polychlorinated biphenyls, potentiated somewhat in nature towards those of higher degree of chlorination. A large number of samples have been examined. Polychlorinated biphenyls are found especially in fish and in sea birds, but also in conifer needles and in some samples of human depot fat. Two hundred samples of soil and water and twenty samples of terrestrial mammals contained no detectable amounts of chlorinated biphenyls. Typical GLC traces are given by Jensen and Widmark (1). By using a nitration method, most of the ordinary pesticides can be removed from extracts and the polychlorinated biphenyl will be unaffected. In this way, it has been shown that two of the major chlorinated biphenyl peaks overlap the peaks of *p,p'*-DDT and *o,p'*-DDT, respectively. This overlap is found with SF-96 columns, most frequently used for the quantitative residue analyses reported in literature, but not with QF-1 columns.

(c) Confirmation of Identity—by K. E. Elgar (Shell Research Ltd., Woodstock Agriculture Research Centre, Sittingbourne, Kent, England)

Multidetection methods would seem to be essential for the analysis of organochlorine residues because of the number of chlorinated components which can be present in any one sample. However, there are problems of interpretation even when techniques are used with the resolution and sensitivity of electron capture GLC. For example, the resolution of the GLC column may not be sharp enough to separate components; or impurities present in the technical pesticide or other natural products present in the extract may complicate the analysis; or some insecticides may thermally or catalytically decompose during the analysis.

For these reasons, residues of organochlorine compounds should be confirmed by other analytical methods. If enough material can be isolated, infrared spectroscopy or mass spectroscopy can give powerful evidence of identity. However, with other methods in common use the physical basis underlying separation is very similar. Robinson *et al.* (2) reported a highly significant concordance between the "p-values" for pesticides in the various partition systems described by Beroza and Bowman (3). Similarly, retention times on different GLC stationary phases and R_f of pesticides in various TLC or paper chromatographic systems are highly correlated. Furthermore, R_f values in paper chromatography are closely correlated with R_f values in TLC and with p-values. It is possible to do a great deal of work with the intention of establishing the identity of a compound, but very little additional evidence will be gained because the results from the methods used were not independent. GLC retention time, an R_f value from TLC or paper chromatography (or a p-value), a GLC retention time of a derivative formed by chemical reaction, and pesticidal activity against a susceptible organism are considered to be independent parameters giving significant evidence of identity.

5. Organophosphorus Insecticide Residue Analysis

The Commission concluded that while the regulatory methods were adequate for parathion and malathion, clarification of the terminal residue situation would be welcome in some instances. However, more work on the cleanup stage for malathion residues in eggs, milk, and dried fruit was indicated.

6. Dichlorvos Residue Analysis

By R. A. E. Galley (Shell Research Ltd., Woodstock Agricultural Research Centre, Sittingbourne, Kent, England)

Five different approaches have been developed for the determination of traces of dichlorvos: total phosphorus (4, 5), colorim-

LEXOLDMON000075
WATER_PCB-00035542

etry using dinitrophenylhydrazine (6), alkaline resorcinol (7, 8), specific bioassay (9), cholinesterase inhibition (10–12), and gas-liquid chromatography with the thermionic detector (13) and the microcoulometric detector (14).

On the basis of sensitivity, selectivity, speed, and possibility of multidetection, the most suitable technique is GLC. Its sensitivity (in the nanogram range with the more sensitive detectors) is approached only by the cholinesterase inhibition procedure, and is unmatched on the other counts. However, there are two aspects in the analysis of traces of dichlorvos by GLC in which improvements are needed. These are the suppression of decomposition of dichlorvos on the GLC column, and the selection of the best detector from (*a*) electron capture, (*b*) thermionic, (*c*) microcoulometric, and (*d*) flame photometric.

The other sensitive method which is in widespread use (and which, together with GLC, offers additional evidence of identity) is the cholinesterase inhibition procedure. This is a general method for anticholinesterases; to analyze mixtures of inhibitors, separation before the analysis is required. However, the volatility of dichlorvos is such that it can be steam distilled from extracts even when they contain a high proportion of fatty material. It is possible, therefore, to obtain a degree of specificity in the analysis for dichlorvos by cholinesterase inhibition by incorporating a steam distillation step before the determination.

Procedures for the collection of dichlorvos vapor from the air, extraction of residues from crops and tissues, and cleanup are available. Dichlorvos may be quantitatively removed from air by bubbling through distilled water. Chloroform, methylene chloride, and benzene have been used to extract dichlorvos residues. Partitioning from an organic solvent into water, column chromatography on silica gel, and steam distillation provide adequate cleanup from fatty and nonfatty materials.

7. Piperonyl Butoxide Residue Analysis

The Commission recognized the need for reliable methods for residues of piperonyl butoxide and certain allied materials such as allethrin and MGK 245.

8. Radioactivation Methods of Analysis

The Commission was informed that the International Atomic Energy Agency (IAEA) had indicated that it would be prepared to (*a*) provide facilities for co-ordinating collaborative studies of existing radioactivation methods, (*b*) assist organizations in the use of activation techniques for the evaluation of analytical methods in general, and (*c*) help laboratories wishing to establish radiochemical techniques (15). It was agreed that while radiochemical methods of analysis were useful for mercury and for bromine residue determinations, they were very expensive and not necessarily more sensitive or more specific than some other methods. Moreover, although they were often useful in metabolic or field residue trial studies, radiochemical methods were in general inappropriate to regulatory methods of analysis; the development of various other methods of regulatory residue analysis was far more suitable.

References

(1) Jensen and Widmark, *Acta Chem. Scand.*, in press.
(2) Robinson, J., Richardson, A., and Elgar, K. F., "Chemical Identity in Ultramicro-Analysis," presented at the 152nd Meeting of the American Chemical Society, September 1966, at New York City.
(3) Bowman, M. C., and Beroza, M., *This Journal* **48**, 943–952 (1965).
(4) Laws, E. Q., and Webley, D. J., *Analyst* **86**, 249–255 (1961).
(5) Saliman, P. M., *Anal. Chem.* **36**, 113–114 (1964).
(6) Hughes, J. T., *Analyst* **88**, 318–319 (1963).
(7) Geiger, M., and Fürer, R., *Z. Anal. Chem.* **174**, 401–407 (1960).
(8) Büchler, W., Heizler, W., and Fürer, R., *ibid.* **213**, 28–30 (1965).
(9) Sun, Y.-P., and Johnson, E. R., *This Journal* **46**, 524–530 (1963).
(10) Giang, P. A., and Hall, S. A., *Anal. Chem.* **23**, 1830–1834 (1951).
(11) Giang, P. A., Smith, F. F., and Hall, S. A., *J. Agr. Food Chem.* **4**, 621–622 (1956).
(12) Porter, P. F., "Vapona Insecticide

MON-MT-008568

LEXOLDMON000076
WATER_PCB-00035543

(DDVP)", in *Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives, Vol. II*, G. Zweig (Ed.), Academic Press, New York, 1964, pp. 561–579.
(13) El-Refai, A. R., and Giuffrida, Laura, *This Journal* 48, 374–379 (1965).
(14) Boone, G. H., *ibid.* 48, 748–752 (1965).
(15) "Radioisotopes in the Detection of Pesticide Residues", proceedings of the Panel on the Uses of Radioisotopes in the Detection of Pesticide Residues, International Atomic Energy Agency, Vienna, April 12–15, 1966.

IUPAC Commission on Terminal Residues

By H. EGAN, *Secretary to the Commission* (Laboratory of the Government Chemist, Cornwall House, Stamford St., London, S.E.1, England)

The first meetings of the above Commission of the Pesticides Section of the Applied Chemistry Division of IUPAC were held in Geneva in November 1966 under the chairmanship of Dr. H. Hurtig. The following account of the proceedings is based on the minutes of the meeting and on the appendixes drawn up by members and associate members of the Commission as indicated.

1. Terminal Carbamate Residue

The commission discussed the present position regarding knowledge of the nature of the terminal residues of carbamates, including carbaryl, as indicated in the evaluations below, and set up a working group to further this knowledge with particular regard to the uses of carbamates in food production.

(a) Evaluation—by J. William Cook (Division of Food Chemistry, Food and Drug Administration, Washington, D.C. 20204)

In the earlier studies on carbaryl residues, both carbaryl and its hydrolytic product, 1-naphthol, were considered. Residue studies on many crops showed that the quantity of 1-naphthol was small and difficult to separate from carbaryl. Thus, it was considered that 1-naphthol need not be measured separately from carbaryl. Tolerances for carbaryl established in the United States are based on the assumption that the major part of the residue is the intact carbamate. Since the establishment of the initial tolerances for carbaryl, work has been done which may have some bearing on the quantitative and qualitative aspects of the residue.

Light, including artificial light and sunlight, changes carbaryl. In the formulated state it slowly degrades under the influence of ultraviolet and sunlight to give several unidentified products (1). Crosby *et al.* (2) reported that ethanol or hexane solutions of some methylcarbamate insecticides, including carbaryl, gave a variety of cholinesterase-inhibiting derivatives when exposed to either artificial light or sunlight. The products were separated but not identified. Abdel-Wahab and coworkers reported that the nature of the surface, the light, and the compound affected the rate of conversion of carbaryl and other carbamates *in vitro* in the solid state.

Since neither the qualitative nor quantitative aspects of photodecomposition of carbamates have been established, the significance of these studies in relation to current and future tolerances for carbaryl must await further study.

Whitehurst *et al.* (4), using the colorimetric method of analysis sensitive to carbaryl, 1-naphthol, and 1-naphthol conjugates, concluded that when cows were fed a diet containing carbaryl, no residues, to the limit of the method, were found in milk. This was

MON-MT-008569

LEXOLDMON000077
WATER_PCB-00035544