CONFIDENTIAL - FILE NOTE
LABORATORY CIRCULATION ONLY

FILE NOTE NO. 0635

CWA/VEC/61

20th July, 1965.

REVIEW OF ANALYTICAL METHODS FOR THE DETERMINATION OF INSECTICIDE RESIDUES

SUMMARY

The insecticides most commonly used on tobacco are DDT, Aldrin, Dieldrin, Endrin, Parathion and Malathion, with MH30 used as a growth regulator. A method for the determination of MH30 has been developed at R. & D.E. but no methods are available for the determination of any insecticides.

A review has therefore been made of the methods available for the determination of pesticide residues which indicates that the most satisfactory methods are those based on a GLC assay, as by this means the pesticides are identified as well as quantitatively determined. Alternatively, identification may be carried out by paper chromatography or thin-layer chromatography followed by a suitable spectrophotometric method for quantitative assay. It is proposed to check which of the methods is the most satisfactory for the determination of pesticide residues on tobacco. When satisfactory methods have been developed R. & D.E. can monitor all tobaccos used by the Company with respect to pesticide residues.

It is suggested that contact is made with the newly formed Pesticide Residue Analysis Information Service (PRAIS) of the Laboratory of the Government Chemist as knowledge of their experience in this field would be invaluable particularly about the supply of pure insecticides to be used as analytical standards.

INTRODUCTION

Great interest has developed recently - outside scientific circles as well as within - in the possible presence of pesticide chemicals on foodstuffs. Some pesticides are now in use for the control of tobacco insects and diseases and as it may be necessary to monitor tobaccos for pesticide residues a review has been made of possible analytical methods.

DISCUSSION

Although there are several hundreds of chemicals which could be used for the control of tobacco insects and diseases, there is no evidence to show that more than a few are actually used. At the moment, DDT, Aldrin, Dieldrin, Endrin, Parathion and Malathion appear to be the most popular. A list of all the pesticides which have been recommended as pesticides for tobacco, is given in the Appendix.

Any method for the determination of pesticide residues falls into three sections, (1) extraction, (2) clean-up and (3) determination.

Extraction

Efficient extraction of the pesticide residue from plant material is often difficult. Many solvents and mixtures of solvents have been studied. Generally emphasis has been placed on using a system which will dissolve the pesticidal chemical whilst remaining miscible with the aqueous medium of the tissue. Mixtures of hexane with iso-propanol have been used extensive (1-6) as well as acetone (7-9) and acetonitrile (10-11). In the case of manufactured tobacco the product is somewhat dehydrated and so solvents such as hexane and dichloromethane would possibly be the most suitable (12-14).

It has been pointed out that care must be exercised not to judge the efficiency of extraction merely on a high recovery of a chemical added during

an analytical procedure. Good recoveries of the chemical added may be achieved when really the degree of extraction of the actual weathered residue is poor (15).

Clean-Up

After extracting the pesticide residue from a sample it is necessary to carry out a clean-up procedure to remove substances present in the extract which would interfere with the subsequent detection and determination.

The ideal clean-up procedure should be applicable to all types of pesticides and extracts. However, because of the complexity of the problem, there is no such procedure and probably never will be. Nevertheless, several clean-up procedures are applicable to a number of different pesticides and these have been reviewed recently (16). Clean-up methods used most frequently employ partitioning between immiscible solvents and/or column chromatography using alumina or carbon. Paper and thin layer chromatographic methods are widely used for screening samples.

Undoubtedly, the clean-up stage is the most laborious and time consuming stage of any analytical method for pesticide residues and so any technique which can do away with this stage is a major advantage. At the moment Neutron Activation Analyses (17) seems to be a possible answer although not a lot of work has been published in this field.

Determination

In the majority of cases, which of the numerous possible pesticides has been used will not be known. A general procedure which can both identify and measure a large number of chemicals at one time is therefore needed. So far, the most useful methods for the determination stage have been chromatographic, mainly G.L.C. Paper or thin layer chromatography are particularly

useful for identification procedures - thin layer having to some extent replaced paper because it is much quicker and has greater resolution and sensitivity.

Spectrophotometric methods, including infra-red, ultra-violet and visible, are still used extensively for quantitative determinations when the identity of the pesticide is known.

METHODS FOR PESTICIDES USED ON TOBACCO

Of the six pesticides most commonly used for the control of tobacco insects and diseases, two are organo-phosphates - Parathion and Malathion whereas the other four are chlorinated hydrocarbons - DDT, Endrin, Aldrin, Dieldrin.

Organophosphate Pesticides

The most satisfactory methods for the determination of organophosphate pesticide residues appear to be those based on the G.L.C. method employing a sodium thermionic detector (STD) which is highly sensitive to phosphorus compounds (18). The response generated in a STD by compounds other than phosphorus compounds is minimal. The hydrogen flame in the detector changes to an intensely blue flame when a phosphorus compound is burned, indicating the formation of a high energy stage, and this mechanism is apparently unique in flame-chemistry. In the presence of sodium, this energy can be measured as electrical energy and is directly proportional to the amount of phosphorus in the flame. As little as 0.1 p.p.m. of Parathion and Malathion can be determined by this method. Chlorinated hydrocarbons, the other group of pesticides used on tobacco do not interfere unless present in fairly large amounts.

Provided that the identity of the pesticide has been proved to be Parathion or Malathion these substances may readily be determined colorimetrically, although a different colorimetric method is needed for each of them.

Parathion contains a nitro-group and the colorimetric method (19) used for its determination involves reduction of this nitro group with zinc dust and hydrochloric acid. The resulting amine is diazotised and coupled with N-1-naphthylethylenediamine. Any other aromatic nitro compounds present must be removed before this determination can be carried out, as must plant pigments, fats and waxes, which interfere with the analyses.

Malathion cannot be determined by the above method as it does not contain a nitro-group. Malathion is decomposed by alkali in carbon tetrachloride--ethanol solution into sodium dimethyl phosphorodithioate and sodium fumarate. The former compound is extracted into water and converted into a copper complex which is then re-extracted into carbon tetrachloride in which it forms an intense yellow colour (19).

Chlorinated Hydrocarbon Pesticides

DDT, Aldrin, Dieldrin and Endrin all are chlorinated hydrocarbons. As was the case for the organophosphates, the best method of analysis is based on G.L.C. as this will identify and estimate the substances present. For this G.L.C. assay, the electron capture ionisation detector is the most satisfactory (20) as it exhibits an exceptionally sensitive and selective response to halogen compound. As little as nanogram (10^{-9} g) quantities can readily be detected.

Alternatively a combined G.L.C.-combustion - micro-coulometric titration procedure (21) can be applied to the analysis of chlorinated pesticides. In this case the sensitivity is less than is the case for the electron capture detector.

Colorimetric methods can be used for these chlorinated pesticides provided a preliminary identification has been carried out. In fact Aldrin, Dieldrin

and Endrin may all be determined by the same colorimetric method. The most widely used is the phenyl azide method (19) although special precautions are necessary due to the explosive nature of the phenyl azide.

The most successful colorimetric method for DDT involves nitration of DDT to the tetranitro derivative (19) which produces a coloured solution in benzene when treated with methanolic sodium methylate.

In conclusion it should be noted that a Pesticide Residue Analysis Information Service (PRAIS) has been established by the Laboratory of the Government Chemist (22) following the recommendation of the Report of the Research Committee on Toxic Chemicals. The main purpose of this service is to assist laboratories by supplying information on methods of analysis for pesticide residues and on their principal degradation and metabolic products. They will also supply information about the supply of analytical standards. As the Laboratory of the Government Chemist has been actively engaged in the analyses of pesticides for some time, any information which they are willing to provide will be extremely useful.

Lora W. Ayers

REFERENCES

1. R.A. Baetz: J. of A.O.A.C. 1964, 47 348.
2. L.J. Hardin and C.T. Sarten: ibid 1964, 47 353.
3. J.C. Maitlen et al: J. Agri Food Chem 1963, 11 416.
4. J.P. Minyard and E.R. Jackson: J.A.O.A.C. 1963, 46 843.
5. H. Human: ibid 1963, 46 195.
6. Van Middelem et al: J. Agri Food Chem 1963, 11 56.
7. W.H. Gutenmann and D.J. Lisk: J. Agri Food Chem 1963, 11 301.
8. J. Kubistova: Pracovni Lekar 1963 15 108; C.A. 1963 59 10682e.
9. A. Taylor et al: Analyst 1964, 89 497.
10. D.E. Coffin and G. Sowary: J.A.O.A.C. 1964, 47 875.
11. R.C. Nelson: J.A.O.A.C. 1964, 47 289.
12. T.E. Archer et al: J. Agri Food Chem 1963, 11 58.
13. M. Chiba and J.H. Morley: J.A.O.A.C. 1964 47 657.
14. J.C. Maitlen et al: J. Agri Food Chem 1963, 11 416.
15. J.W. Cook and S. Williams: Anal Chem 1965, 37 (5) 130R.
16. W.P. McKinley et al: J.A.O.A.C. 1964, 47 (5) 853.
17. R.A. Schmitt and G. Zweig: J. Agri Food Chem 1962, 10 481.
18. L. Giuffrida and F. Ives: J.A.O.A.C. 1964, 47 1112.
19. Analytical Methods for Pesticides, Vol. II, Zweig.
20. J. Burke and L. Giuffrida: J.A.O.A.C. 1964, 47 325.
21. J. Burke and L. Johnson: ibid 1962, 45 348.
22. Chemistry in Britain 1965, 1 (7) 342.

APPENDIX

Common or trade names of pesticide compounds recommended for the control of tobacco insects and diseases are listed alphabetically.

Aldrin

BHC

DDT

DDVP

Demetan

Dieldrin

Dimethoate

Endrin

Guthion

Heptachlor

Malathion

Maneb

Menazon

Metaseptex

Parathion

Sevin

Telodrin

Thiodan

TDE

Penar

MH30

Distribution

Dr. D.G. Felton/File 61
Dr. I.W. Hughes
D.J. Wood, Esq.
Dr. C.W. Ayers