trometer also provides some quantitative information. An ionization chamber-electrometer system can be used in series to detect C^{14} or tritium-labelled components (2,7); ultraviolet or infrared spectrometers can monitor effluents at given wavelengths (6,8), the far-ultraviolet region being most sensitive.

An electron capture detector (12,13), also known as an electron absorption detector, can be used along with a conventional ionization detector to pick out components with high electron absorptivity. At ionization potentials of 15–20 v only the highly halogenated compounds, nitro-compounds, quinones, conjugated carbonyls, and other highly conjugated systems show any change in the ionization current. Ordinary fatty acids and methyl esters are completely unaffected. The method has its origin with electron affinity spectroscopy first reported by Lovelock and Lipsky (14). Expensive instrumentation is not always necessary. The chemical nature of each eluted component can be determined by passing the outlet stream into a number of tubes containing functional group reagents (21).

Identification by Analysis of Isolated Components

The third category is the isolation of fractions and their subsequent analysis by chemical and physical means. In such cases the gas chromatograph is often used as a means for separating mixtures. Nevertheless, collected fractions can be chemically modified and reinjected into the gas chromatograph, thereby establishing the chemical nature of the fraction. For example, an unknown component can be collected and selectively and quantitatively hydrogenated (17) and reinjected to establish the degree of unsaturation and the backbone structure of the unknown. Controlled degradation (10) of the same collected fraction followed by reinjection of the products may elucidate the position of unsaturation.

ACKNOWLEDGMENT

Technical advice by E. P. Jones and I. A. Wolff.

REFERENCES

1. Bagby, M. O., C. R. Smith, Jr., T. K. Miwa, R. L. Lohmar, and I. A. Wolff, J. Org. Chem. *26*, 1261–1265 (1961).
2. Dutton, H. J., JAOCS *38*, 631–636 (1961).
3. Dutton, H. J., *Ibid. 38*, 660–664 (1961).
4. Evans, C. D., P. M. Cooney, and E. J. Panek, *Ibid. 39*, 210–213 (1962).
5. James A. T., J. Chromatog. *2*, 552–561 (1959).
6. Johnstone, R. A. W., and A. G. Douglas, Chem. and Ind. (London), 1959, 154.
7. Jones, E. P., L. H. Mason, H. J. Dutton, and R. F. Nystrom, J. Org. Chem. *25*, 1413–1417 (1960).
8. Kaye, W., Anal. Chem. *34*, 287–293 (1962).
9. Kovats, E., Helv. Chim. Acta *41*, 1915–1932 (1958).
10. Lemieux, R. U., and E. von Rudloff, Can. J. Chem. *33*, 1701–1709 (1955).
11. Lindeman, L. P., and J. L. Annis, Anal. Chem. *32*, 1743–1749 (1960).
12. Lovelock, J. E., *Ibid., 33*, 171–172 (1961).
13. Lovelock, J. E., Nature *189*, 729–732 (1961).
14. Lovelock, J. E., and S. R. Lipsky, J. Am. Chem. Soc. *82*, 431–433 (1960).
15. Miwa, T. K., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal Chem. *32*, 1739–1742 (1960).
16. Miwa, T. K., and I. A. Wolff, JAOCS *39*, 320–322 (1962).
17. Ogg, C. L., and F. J. Cooper, Anal. Chem. *21*, 1400–1402 (1949).
18. Smith, C. R., T. L. Wilson, and K. L. Mikolajczak, Chem. and Ind. (London), 1961, 256–257.
19. Smith, C. R., T. L. Wilson, T. K. Miwa, H. Zobel, R. L. Lohmar, and I. A. Wolff, J. Org. Chem. *26*, 2903–2905 (1961).
20. Smith, J. F., Chem. and Ind. (London), 1960, 1024–1025.
21. Walsh, J. T., and C. Merritt, Anal. Chem. *32*, 1378–1381 (1960).
22. Wehrli, A., and E. Kovats, Helv. Chim. Acta *42*, 2709–2736 (1959).
23. Wolff, I. A., and Q. Jones, Chemurgic Dig. *19*(7), 4–9 (1960).
24. Woodford, F. P., and C. M. van Gent, J. Lipid Research *1*, 188–190 (1960).

[Received November 12, 1962—Accepted March 14, 1963]

Assay of Insecticides and Herbicides in Fats and Oils

J. W. COOK, Division of Food, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

Abstract

Types of residue and methods data required in the establishment of pesticide tolerances in food products is discussed. Emphasis will be on those products and methods involving fats and oils.

A summary of some of the current research efforts in FDA in methodology for pesticidal residues in food products is given. These methods are in the nature of screening or sorting techniques which identify and determine a number of different chemicals in a single analysis. Adequate separation of these chemicals from the fats, oils, and waxes of food products is often the most difficult step in methods development.

Introduction

MANY of the insecticides, herbicides, and other pesticides are present in food products in very low concentration. They must be purified and concentrated before an analysis can be made. Fats, oils, and waxes contribute a good deal to the complexity of our problems of analysis for pesticide residues, because the extraction procedures used to remove many of these chemicals from plant and animal products, also extract the fats, oils, waxes, and similar products. This extraction step is usually simpler to perform than the second step, which is to separate the minute quantities of pesticide chemicals from the relatively large amount of fats, oils, or waxes. Almost invariably these substances interfere with the final or determinative step in the analysis for the pesticide. Therefore, the problems of assay of fats and oils are much the same as those encountered in the assay of foods generally.

The general subject of pesticide chemicals has not been a major item of discussion at AOCS meetings; this paper is in general rather than specific terms, and is divided into two parts. First, is a discussion of some general considerations which relate ultimately to assay or methods. Secondly, some developments in the methods field are described. The discussion on analysis is limited mainly to work in the laboratories of FDA.

Pesticide chemicals include many substances other than insecticides and herbicides. The discussion is not restricted to the topic title as analytical problems of one often pertain to the other, and the physicochemical procedures used does not discriminate between the various biological effects of chemicals. The term "pesticide chemical" is defined by the Federal Insecticide, Fungicide, and Rodenticide Act. Included are those chemicals which control insects, weeds, rodents, nematodes, fungi, spiders, and those which act as plant growth regulators, defoliants, desiccants, etc. Recently, (March 27, 1962), this was further expanded to include, as pests, mammals, birds, fishes, snakes, invertebrates, roots, and viruses. We have established tolerances for about 125 chemicals and there are 200–

300 other chemicals registered for use on agricultural crops on a no residue basis.

Some comments on the rules under which tolerances are set will give a clearer insight into some of the problems in this general area.

Discussion

The Food, Drug, and Cosmetic Act of 1938 stated that no poisonous or deleterious substance could be added to food unless it was required in the production of the food, or could not be avoided in good manufacturing practice. The law gave FDA the authority to set safe tolerances for those pesticide chemicals required in crop production. However, the tolerance-setting mechanism authorized by that Act was cumbersome, and establishment of official tolerances under it was slow and difficult. That system was based on public hearings in which any interested person could present any relevant fact. One such hearing was held in 1950. It lasted for about six months. Some of the tolerances set from that hearing were not established with all of the same background and objectives in mind that exist today. Not as much data on methods of analysis, nor as much residue data, were required at that time as are required now.

The exclusive concern was with toxicity considerations in establishing tolerances. Since 1954, operation has been under the Miller Amendment; the established tolerance now more closely reflects a residue level which will not be exceeded by a useful, practical application rate, as well as a level no higher than necessary, and a level which toxicity studies show to be safe. Also, no numerical tolerance is set unless there is a method of analysis with which to enforce the tolerance. Some "zero tolerances" have been set which created some enforcement methods problems. With the introduction of each new chemical, a sponsoring company almost invariably asks the question: How sensitive a method do we need to show that there is a zero amount of residue? In every case, the pharmacologists have been consulted to ascertain the sensitivity required and the questioner has been told what level of sensitivity was thought necessary in light of the available knowledge of the pharmacology of the chemical. Of course, it is recognized that a method of analysis seeks to show the presence rather than the absence of a substance, and therefore, in effect amounts up to the sensitivity of the method are ignored. In this work, it is considered that a zero tolerance has been met where no residue is detected by a method of suitable sensitivity. This is believed to be scientifically sound, and protects the public health; yet it does not unrealistically attempt to establish a zero amount in an absolute sense.

The Miller Amendment provided for the establishment of tolerances for pesticide chemicals on raw agricultural products. It did not provide for the establishment of tolerances for pesticide chemicals in a processed food. This gave some trouble. For instance, chemicals which concentrate in a specific fraction of a food, such as in the fat or oil, could have resulted in violative by-products, the fat or oil being the by-product. The U.S. producers of olives did not have any difficulty with parathion, but the European producers did. They had an insect problem which, it was said, could only be controlled at that time by late season application of parathion. The rate of application was such that about 1 ppm remained in the harvested crop. This was the level at which had been set a number of tolerances, and it probably would have been acceptable as a general tolerance for parathion for olives in the U.S. However, all the parathion was in the oil, so when it was pressed out, about 3–4 ppm was present in the finished oil. Obviously, any tolerance set for the fresh olive would be exceeded by the residue actually in the oil. The Food Additive Amendment permits a petitioner to request a tolerance in the processed food which is higher than that on the raw agricultural product if such request reflects good manufacturing practices and the tolerance requested is safe.

Under the Miller Amendment some tolerances have been set for residues in the fat of meat of certain animals. It is not as easy to describe the condition or time when a steer or part thereof is actually a raw agricultural commodity as it is in the case of a head of lettuce. It was recognized that the residues of many chemicals would be present in the fat, so the tolerances were set for such chemicals in meat products on the basis of the residue in the fat. This enabled tolerances to be set in a practical, legal, and safe way and eliminated the difficulty of attempting to relate the residue to any particular cut of meat or portion of the carcass or the whole steer, which would be difficult to do.

Before any tolerances are set under the Miller Amendment, a great deal of information and data is obtained from the sponsoring company. These data include reports on chemistry, utility (evaluated solely by USDA), pharmacology, identity of residue, method of analysis, and amount of residue expected. All of these data are studied and evaluated carefully, always considering safety to the consumer and practicality of the tolerance.

Sometimes much work must be done to establish identity of the toxic residue, particularly when the toxic residue is different from the parent compound. The conversion of the parent chemical to other toxic residues is not uncommon and has created one of the most difficult problems of residue determination. These conversion residues very often have quite different chemical properties from the parent.

After the identity of the residue is established, a specific method is needed to determine that residue in the presence of other residues expected to be present. A method must be available to police the tolerance. For instance, radiotracer type work may be acceptable to show the type and level of residue, but it could not be used to enforce a tolerance set from such data. So, if some data for establishing the tolerance are obtained by a radiotracer technique, there must be an additional method for enforcement purposes. Also, a general method such as total organic chlorine may be adequate to assist in establishing a tolerance for a compound when it is known what chemical has been used, but it is not useful for enforcement purposes on samples of unknown or uncertain history.

All of this produces a large amount of difficult work. However, it is all necessary at the present state of development. It is hoped that it may be possible by use of some of the techniques described below, or other similar techniques, to be able to identify and determine residues more easily. Some of the techniques may assist in reducing the amount of development work required of a sponsoring company which would also reduce the amount of work required to evaluate a petition for tolerance. The work described involves screening or sorting types of techniques. There is another important purpose for this work. In order to enforce established tolerances it is necessary to do

a certain amount of objective sampling of food products in interstate commerce. No history of the chemical treatment of such samples is available. As many as 30 chemicals are registered for use on certain crops on a tolerance basis, and many others are registered on a no residue basis on the same crops. Also, there could be misuse of a number of chemicals with no tolerance or registered use. It is impractical to determine whether such crops comply with the law by following each specific method for each of the possible compounds. The screening or sorting methods are designed to locate, identify, and determine members of a group of compounds simultaneously.

The screening procedure uses many different techniques. At present trial is made of paper chromatography, column chromatography, gas chromatography, partitioning, polarography, infrared analysis, and bioassay. None of these individually can give complete answers, but combinations of these techniques give more than adequate procedures for certain groups of compounds. There is hope that these successes will be extended to other groups of compounds in the future.

Encouraging success has come from a combination of column chromatography followed by partitioning between certain solvents, then making the final determination by a number of modifications of paper chromatography and/or gas chromatography. This success was obtained with chlorinated pesticides. The combination of techniques is called the Mills Procedure (1). First, the material is extracted with hexane-ethanol; this removes, of course, much of the fat, oil, wax, and other related products, as well as many of the pesticide chemicals. Evaporation of the solvent mixture leaves a residue which is where the problem of analysis of residues in fats and oils begins. The residue is taken up in petroleum ether. This crude extract is cleaned up by passing the solution through a specifically and highly activated Florisil column. Much of the fats, waxes, and oils are held on the column as are the pesticide chemicals. However, many of the pesticide chemicals are eluted from the column by the use of 6% ethyl ether in petroleum ether, whereas most of the plant extractives are not eluted. Fifteen percent ethyl ether in petroleum ether is required to remove dieldrin and/or endrin from the column. This concentration of ethyl ether very often does elute some plant material, so this eluate is usually cleaned further by partitioning between petroleum ether-acetonitrile. Many of the interfering materials remain in the petroleum ether and the pesticides go into the acetonitrile. The acetonitrile is diluted with water and then shaken again with petroleum ether, and the pesticides partition back into the petroleum ether. Generally, very good results by paper chromatography or gas chromatograpy can be obtained on extracts cleaned up in this way. Fly bioassay can also be used. When this procedure is to be applied directly to fats and oils, it is necessary to go through the acetonitrile-petroleum ether partitioning before the Florisil partitioning. Some more recent work has shown that plant material can be extracted directly with acetonitrile, thus shortening the procedure.

Figure 1 shows a paper chromatogram of the more common pesticides which are identifiable by this procedure, and Figure 2 shows a chromatogram of some pesticides which were added to butterfat and recovered by the above technique. Figure 3 is a gas chromatogram obtained by use of a microcoulometric gas

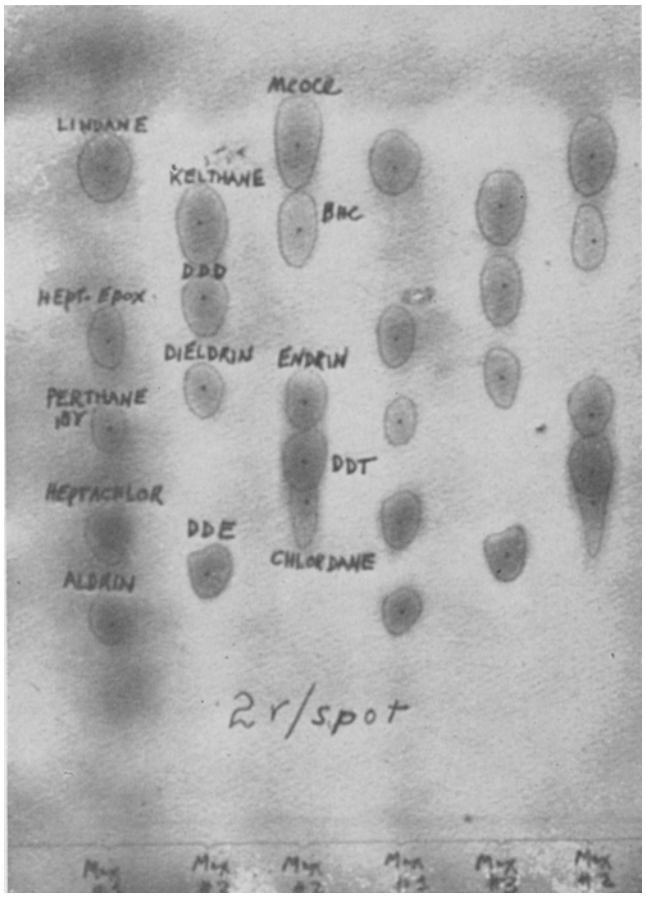

FIG. 1. Paper chromatogram which shows some of the pesticides determinable by the Mills procedure. The three rows of spots on the right half are duplicates of the three rows on the left half.

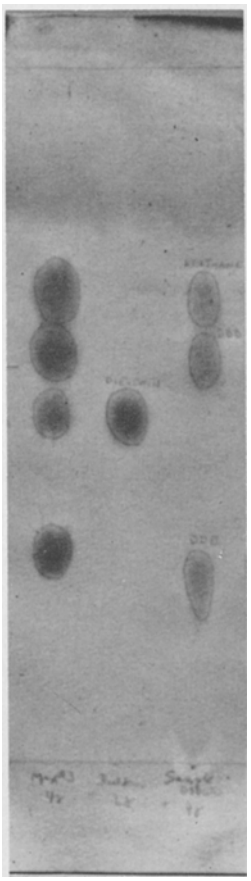

FIG. 2. Paper chromatograph showing recovery of pesticide added to milk. Left row are standards, 4 mmg each of DDE, dieldrin, DDD, and kelthane reading from bottom to top. The middle one is 6 mmg of dieldrin and the right column shows compounds recovered from milk. Dieldrin is recovered by a different system than is illustrated here.

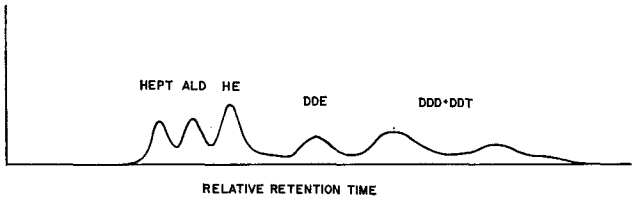

FIG. 3. Chromatograph showing recovery of some pesticides added to butter, following procedure described in text.

chromatographic apparatus showing the results obtained from a sample of butterfat to which pesticides had been added and then recovered by the Mills procedure.

Recently some associates working on food additives suggested that it may be possible to improve the above procedure by substituting dimethyl sulfoxide for acetonitrile. Eidelman, in this laboratory, has investigated the use of this solvent with excellent results so far. This was not a direct substitution of DMSO for acetonitrile, but required a good deal of adjustment of conditions and solvents. The report on this work will be available soon (2). About 5 g is the upper limit of fat that can be handled by the acetonitrile-petroleum ether partitioning procedure. The limits of sensitivity for the location of the spots on the chromatogram is, for example, about 0.5 μg of DDT. Therefore, about 0.1 ppm is the lower limit of sensitivity of the method for DDT in butterfat. Much experience is required for confidence at this level. However, with the use of DMSO it is possible to recover quantitatively pesticides from 100 g of butterfat so that all of the pesticides can be spotted; therefore, the sensitivity is increased about 20 fold. Thus, on a fat basis .005 ppm of DDT can be detected. On a whole milk basis this calculates to about .0002 ppm or .2 ppb.

Figure 4 shows a chromatogram of a sample of

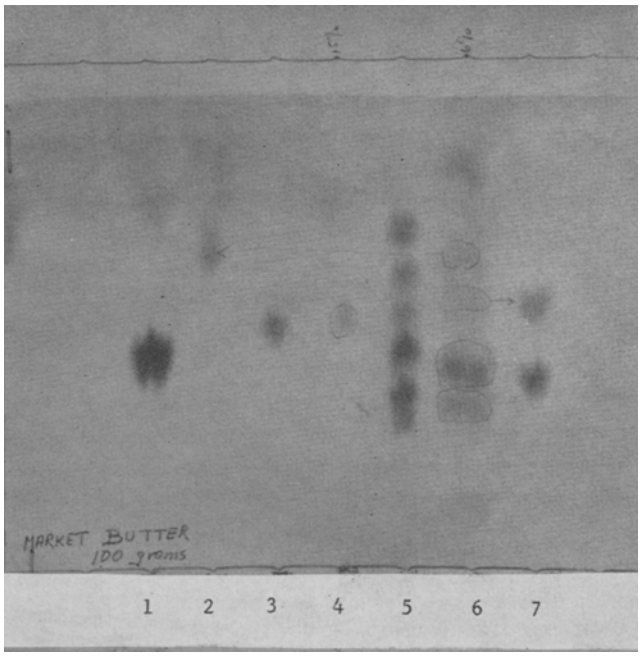

FIG. 4. Paper chromatograph showing residues found when 100 g butter were processed by DMSO method. (1) 6 mmg DDT standard; (2) 2 mmg BHC standard; (3) 2 mmg endrin standard; (4) residue eluted from Florisil by 15% ethyl ether in petroleum ether; (5) 2 mmg each of aldrin, DDE, DDT, dieldrin, TDE, and lindane as standards (reading from bottom to top); (6) residues eluted from Florisil by 6% ethyl ether in petroleum ether; and (7) 2 mm each of heptachlor (below) and heptachlor epoxide (above) standard.

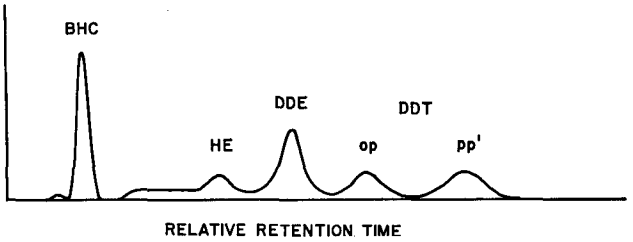

FIG. 5. Gas chromatograph of market butter using DMSO cleanup. This shows the same pesticides as Figure 4 except that dieldrin is missing. It does not elute from Florisil column with the other pesticides.

butter cleaned up by the DMSO procedure. Use of this highly sensitive procedure shows that this sample contained DDT, DDE, lindane or BHC, heptachlor epoxide, and dieldrin. Each of these are present at about .02 ppm on a fat basis. Calculating this to the whole milk basis, each compound is present at a level of about 1 ppb. Figure 5 shows a gas chromatogram of this same cleaned up butter. It is unequivocal chemically that this sample of market butter has these small amounts of pesticides. These values are a great deal below the pharmacologically significant figure for some of the chlorinated compounds, but it is not so far under the value needed to be ascertained for certain others of this group. It is gratifying to have this sensitivity in our analytical method because it gives greater assurance for the detection of significant quantities. It is no longer necessary to wrestle with the problem of chemical identity or chemical significance of the compound in question at the pharmacologically significant level range.

The Mills procedure was originally developed with butterfat, and about 5 g of butter oil was all that could be handled; however, the procedure worked well for many fruit, vegetable, and other products as long as the fatty substances extracted from the plants did not exceed 5 g. An attempt to use the Mills procedure for other types of oils, disclosed substances not recognized and, therefore, further study

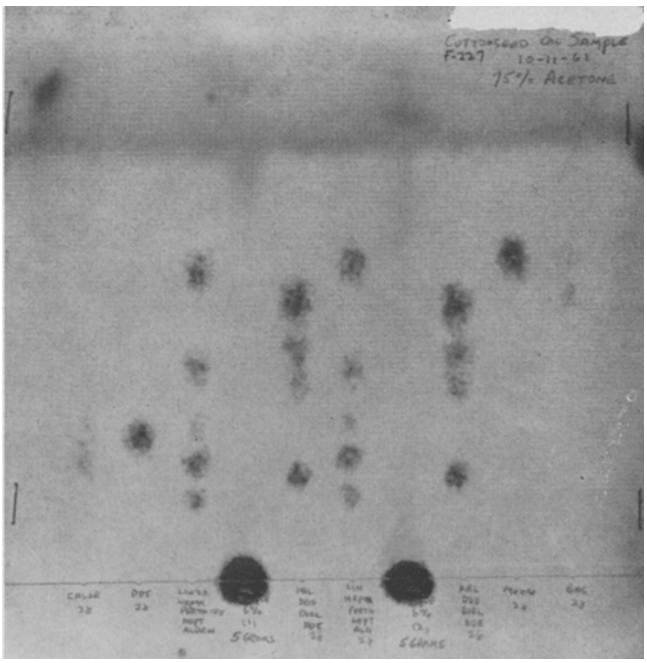

FIG. 6. Paper chromatograph obtained from cottonseed by the Mills procedure. All spots are standard pesticides except the large spots at the origin, which are caused by unknown material.

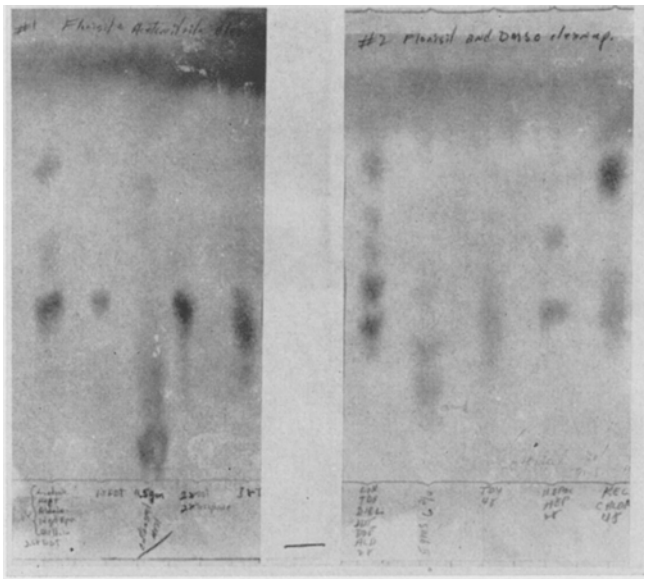

FIG. 7. Comparison of paper chromatographs obtained from cottonseed meal by both the Mills (left) and the Eidelman procedures (right). On the left chromatogram the middle spot is from the meal, and on the right chromatogram the second column is from the meal. All others are various standards.

is necessary. Some of these materials are shown in Figures 6–11. Figure 6 shows a large spot at the origin obtained by the usual Mills procedure from cottonseed oil. The oil from a cottonseed meal was extracted with acetonitrile in the usual fashion (using Florisil) and chromatographed. The results obtained are shown on the left of Figure 7; there is a large spot which reacts to silver like the chlorinated pesticides; also there is a streak which appeared to be toxaphene. When this same sample was treated by the DMSO-Florisil procedure, a streak was present which was displaced from toxaphene as shown on the right side of Figure 6. When this material was gas chromatographed, the graph shown on the lower half of Figure 8 was obtained. This is complicated and difficult to interpret. The peaks appear to be the following: (The figures in parentheses are the relative retention times with aldrin assigned the value of 1.00.) Left to right, lindane (.54); unknown (.65); heptachlor (.88); unknown (1.10); heptachlor epoxide (1.27); α chlordane (1.46); perthane (1.81); toxaphene and op DDT (2.02); pp′ DDT (2.40); toxaphene (2.7); toxaphene (3.11). If these pesticides are all present, they are in the fraction of a ppm range. The upper half of the figure is a graph of toxaphene.

An attempt was made to use the Mills procedure for the analysis of eggs for chlorinated pesticides. Figure 9(a) shows a typical paper chromatograph obtained from some egg samples for which there was no sample history. Again a streak of material was obtained which precipitated silver like the chlorinated

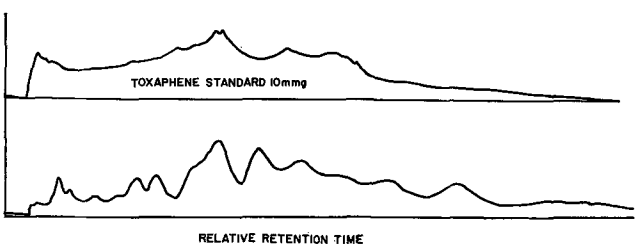

FIG. 8. Gas chromatographs comparing the halogenated material from cottonseed meal below with a toxaphene standard above. See text for possible explanation of peaks.

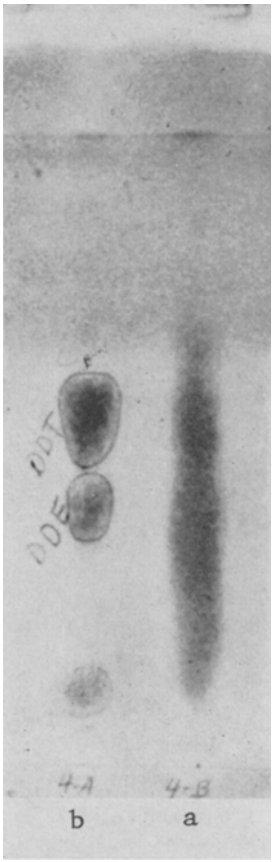

FIG. 9. Paper chromatogram showing halogen spot test (a) before and (b) after treatment of egg extract with acetone. In column (b) the top spot is DDT, the middle one is DDE, and the bottom one is probably from reagents.

pesticides, yet the extract was not as toxic to flies as would be expected if all of this were a typical chlorinated pesticide. It was found that if the residues were treated with acetone a portion was precipitated and then spots typical of some of the chlorinated pesticides were obtained as shown in Figure 9(b).

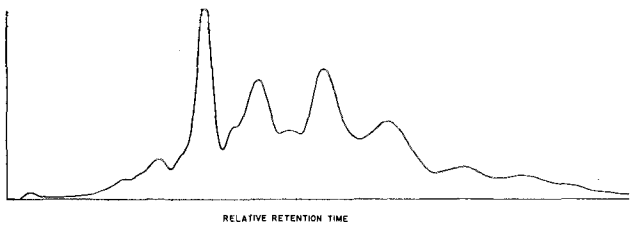

FIG. 10. Gas chromatograph obtained from same extract as in Figure 9(a).

Figure 10 shows a gas chromatograph of the halogenated products before acetone treatment and Figure 11 shows the curves obtained after the acetone treatment. It seems clear that this sample of egg was contaminated with some DDT and DDE.

These illustrate some of the accomplishments and some of the difficulties with methods for pesticides in fats and oils.

It is regrettable that for other groups of pesticide compounds systems of analysis have not been devel-

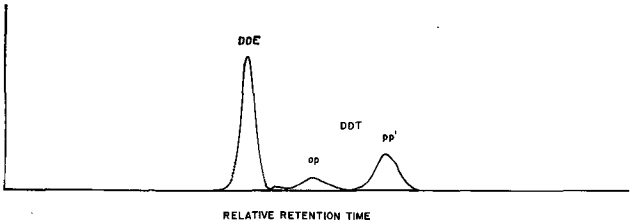

FIG. 11. Gas chromatograph obtained from same extract as 8(b).

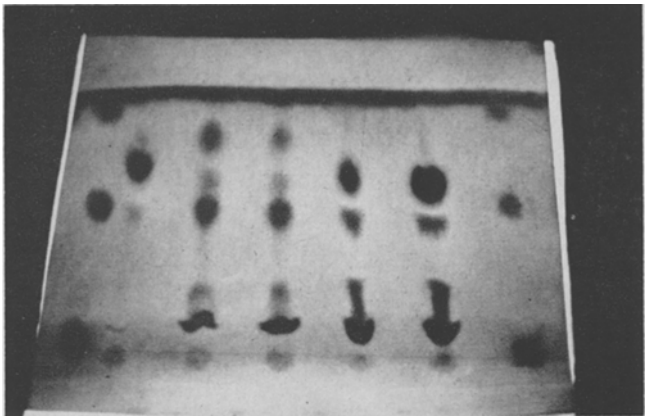

FIG. 12. Paper chromatograph showing results obtained from kale treated with Di-Syston when analyzed by Getz procedure.

oped to the same state of usefulness as that of the Mills procedure for the chlorinated ones. The FDA is working intensively on systems for organophosphates, but these compounds present some more difficult problems than the chlorinated compounds. Whereas all the chlorinated compounds discussed in this paper are relatively highly fat soluble, only some of the organophosphate compounds are fat soluble whereas others are highly water soluble and others have intermediate solubilities. To further complicate this picture, at least from an analytical point of view, many of the fat soluble ones change in or on the plants or animals to more water soluble compounds which are highly significant pharmacologically.

Figure 12 shows a chromatogram done by Getz in FDA laboratories and illustrates some of these changes that take place with some phosphate compounds (3). The spots near the bottom are fat soluble whereas those toward the top are water soluble. This chromatogram shows the changes that take place when Di-Syston is sprayed onto kale plants and the plants are sampled at various times. From left to right

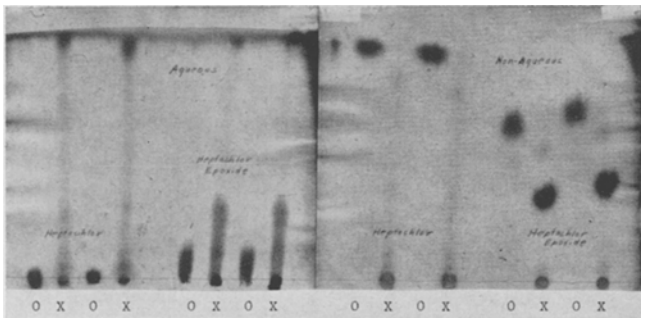

FIG. 13. Paper chromatograms showing effect of ultraviolet light on some chlorinated pesticides. The left chromatogram was developed by Mitchell's aqueous system, and the right one was developed by his non-aqueous system (4). On each chromatogram the four columns on the left half are heptachlor and the four columns on the right half are heptachlor epoxide. O denotes not exposed to UV light; X denotes exposed to UV light.

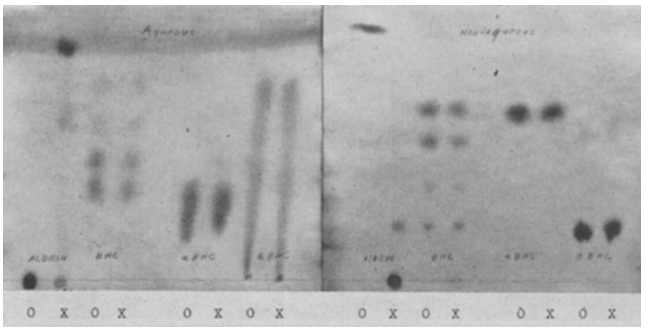

FIG. 14. Paper chromatogram showing effect of ultraviolet light on some chlorinated pesticides. The left chromatogram was developed by Mitchell's aqueous system, and the right one by his non-aqueous system (4). On each chromatogram the first two spots on the left are aldrin, the next two BHC, the next α BHC, and the last pair β BHC. O denotes not exposed to UV light; X denotes exposed to UV light.

the spots represent the conditions at 0, 2, 4, 4, and 15 days after application. It can be seen that a number of compounds form which are much more water soluble than Di-Syston. On each margin at origin is a spot of Di-Syston, the two higher spots on each margin are from Systox thiol sulfone and thiol sulfoxide, each of which is much more water soluble than is Di-Syston.

It is known that some of these phosphates do persist as residues in fats of animals and some oils, and surely must persist to some degree in the fat and oil portions of plants. But there is no system of analysis yet which will show the whole picture.

Figures 13 and 14 show two chromatograms illustrating some other work being done at FDA which may be of significance to fats and oils but as yet is incomplete. Mitchell has been studying the effect of UV light on spots of the chlorinated compounds on paper (4). He has spotted some 150 different compounds and chromatographed them with and without exposure to UV light for a short while. Figure 13 shows the effect obtained using heptachlor and heptachlor epoxide. The left chromatogram was chromatographed by Mitchell's aqueous system and the right one by his non-aqueous system. Obviously there is a marked effect on these compounds. Note that these conditions do not convert heptachlor to heptachlor epoxide and also that heptachlor epoxide does convert to some other compound.

Figure 14 shows aldrin and BHC. Note that aldrin is changed markedly but BHC isomers are unaltered. It is hoped to discover whether these are of significance in practical usage of the chemicals on plants, or whether they change only under the condition to which they were subjected in this experiment.

REFERENCES

1. Mills, P. A., J. Assoc. Offic. Agri. Chemists *44*, 171 (1961).
2. Eidelman, Martin, *Ibid. 45*, 672 (1962).
3. Getz, Melvin, *Ibid., 45*, 393 (1962).
4. Mitchell, Lloyd, *Ibid. 44*, 643 (1961).

[Received November 12, 1962—Accepted March 14, 1963]

• *Erratum*

JAOCS, *40*, page 127, HASHIMOTO et al.: ON THE STRUCTURE OF HIGHLY UNSATURATED FATTY ACIDS OF FISH OILS BY HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS. In the footnotes to Table I, "Ratio B: The proton number of the mixed acid esters having one divinylethane and divinylmethanes," should read: Ratio B: The proton number ratio of the mixed acid esters having one divinylethane and divinylmethanes.

Listing in the Index of the same issue, page 2, should read: On the Structure of Highly Unsaturated Fatty Acids of Fish Oils by High Resolution Nuclear Magnetic Resonance Spectral Analysis, by Tetsutaro Hashimoto, Kenkichi Nukada, Hisako Shiina, and Tomotaro Tsuchiya.