PERSISTENT PESTICIDES

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Compounds of many different classes are in use for the control of various plant pests or vectors of disease. In recent years, increasing attention has been given to the epidemiological and ecological significance of these compounds, and one class of compounds, namely the organochlorine insecticides, has been the subject of growing criticism on the grounds that the so-called side effects are really of serious ecological significance. The effects are generally attributed to two characteristics of these compounds: their stability (and consequent persistence in the environment), and their ability to induce increased enzyme activity in the microsomal fraction of the liver (and perhaps of other tissues).

This review will discuss the evidence regarding the residues of these compounds in the general human population, the dynamics of the uptake, storage, and elimination of these compounds in man and other vertebrates; their effects upon hepatic microsomal enzymes; and the metabolites found in vertebrates.

RESIDUE IN MAN

Numerous surveys of the concentrations of organochlorine insecticides in the adipose tissue of members of the general population of various countries have been published; the distribution of residues in the populations of the United States and Great Britain having been most closely studied (1, 2, 3). The reliability of the results of these surveys as estimates of the residues in the general population depends upon the sampling and analytical procedures that have been used.

Sampling techniques.—The sampling procedures may be considered under two main aspects: first, the method of selection of persons from whom specimens of adipose tissue were taken; and second, the anatomic site from which the specimen was taken. The first point is obviously of critical importance if the values found are to be used to obtain valid estimates of the average concentration and variance of the concentration of an insecticide in the adipose tissue of the general population. In the reports of most surveys there is, unfortunately, a lack of detailed information on the method of selection of persons from whom specimens were collected. Statements, for example, that the specimens were obtained during routine surgery or au-

topsies are often the only information given. In the reports on most surveys it is also stated that the persons had had no known occupational exposure to organochlorine insecticides. About 50 per cent of the published surveys give results of the analyses of necropsy adipose tissue, necropsy and biopsy specimens were analysed in about 30 per cent of the surveys, and biopsy specimens in about 20 per cent of the surveys. The proportions of the numbers of specimens collected from the two sexes vary considerably from one survey to another; age distribution is also quite variable, and the ethnic composition may also vary. The effects of these different variables are discussed below.

Necropsy and biopsy specimens.—Direct comparisons of the residues found in necropsy and biopsy specimens of adipose tissue have been made in a few surveys. Dale, Copeland & Hayes (4) concluded that there were no significant differences between the concentrations of DDT-derived material in the two types of specimens collected from people in India, and a similar conclusion was reached in a survey of residues of these compounds in Israel (5). Robinson et al. (6) concluded that the mean residues of both DDT-derived materials and HEOD[1] in both types of specimens were not significantly different although the variance of the concentrations in necropsy specimens was greater than that in biopsy specimens.

Residues in persons dying from certain diseases.—A comparison which is related to that between necropsy and biopsy specimens is that of the residues in specimens of adipose tissue from persons who have died from different diseases. Residues of DDT-derived materials were not found to be related to the cause of death in two surveys (6, 7), neither were those of HEOD (6). On the other hand, Radomski et al. (8) and Casarett et al. (9) found differences between the residues in some tissues of some types of terminal patients as compared with those in healthy adults.

Men and women.—No significant difference in the concentrations of DDT-derived material in the adipose tissue of men and women was found in some surveys (4, 5, 6, 10, 11), whereas significant differences were reported in another survey (12). Residues of HEOD in males tend to be higher than in females (6, 12, 13, 14, 15). Surveys of DDT-type compounds in adipose tissue in Florida (16, 17) indicated differences between coloured men and women but not between noncoloured men and women, whereas HEOD showed no sex difference for either ethnic group. Residues of DDT-derived materials in males in Chicago (7), were higher in men than in women, but no sex difference was found in the case of benzene hexachloride. Hayes et al. (18) reported that residues of β-BHC and pp′-DDT were higher in women in New Orleans than in men.

[1] The insecticide dieldrin contains not less than 85 per cent of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene; HEOD is formed by the epoxidation (biological or chemical) of HHDN, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene, the major constituent of aldrin.

Relationship of residues to age.—Several investigations of the variations of residues with age have been made. Residues of DDT-derived materials have been detected in the adipose tissue of prematurely born babies and stillbirths (9, 15, 19–21). Analytically detectable residues of HEOD (15, 20, 21) and BHC isomers (15) have also been reported. These findings are not surprising in view of the physico-chemical properties of the organochlorine insecticides and the results of studies with experimental animals (22–25). Residues of DDT-type compounds have been found in neonates (9, 15, 19, 20, 26, 27), and the concentrations of HEOD (15, 20) and BHC isomers (15) have also been measured. Abbott et al. (15) suggested that the concentrations of organochlorine insecticides in infants tend to decline during the first three months after birth and ascribed this decrease to the rapid increase in the fat content of infants during this period. Residues of DDT-type compounds in infants in Israel also show a tendency to decline in the first few months after birth (19) although the residues in some males appear to deviate from this pattern. After the decline in the first few months residues tend to increase up to about the age of 10 years, the concentrations then remain relatively constant in the various age groups (5, 6, 11, 12, 19), although in some surveys there appear to be small differences between people under 39 and those over 40 years of age (7, 14, 15).

Miscellaneous factors.—Hayes et al. (11) concluded that the residues of DDT-derived materials in vegetarians were lower than in meat-eaters. The difference may be related to the tendency for residues in animal fats to be higher than in other components of the diet. However, it is also necessary to consider the possibility that the difference between these two groups does not arise solely from the differences in their dietary habits. Exposure in the domestic environment to organochlorine insecticides may also affect the residues found in man. Thus, it is noteworthy that the highest residues of DDT-derived materials were found in specimens of adipose tissue collected in Delhi, India (4). The majority of people in this survey were presumably vegetarians and the relatively high residues may be the results of the use of DDT in public health schemes for the eradication of malaria; the use of DDT in grain storage may also be a contributory factor. Radomski et al. (8) also found a correlation between the domestic usage of formulations containing DDT and the concentration of DDT-derived materials in adipose tissue. Residues from people from different social classes do not appear to have been studied, although it is feasible that dietary habits related to socio-economic factors may affect the concentrations of these compounds in the tissues. The residues in people from different ethnic groups have been studied in the United States. Higher residues of DDT-type compounds have been found in nonwhites as compared with whites (7, 9), although no similar difference was found in the case of HEOD (9). The concentration of HEOD in the tissues of volunteers given known daily doses of this compound was inversely related to the adiposity of the subjects (28), and a similar conclusion was reached in the case of dogs given daily doses of

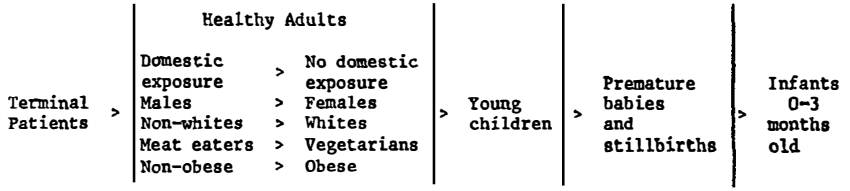

FIG. 1. Residues of organochlorine insecticides in different groups of the general population.

HEOD (29). Thus, the obesity of the persons from whom specimens were obtained may affect the results, and these findings may be pertinent to the general tendency for residues in men to be higher than those in women (see above) since the adiposity of women is greater than that of men (30). It is apparent that the concentrations of organochlorine insecticides in adipose tissue may be affected by a number of factors. The general conclusions that may be drawn from the published surveys are summarised in Figure 1.

The variety of the factors affecting the residues in the adipose tissue of different people has important consequences in relation to inferences drawn from population surveys with regard, for example, to variations between different geographical areas, times of collection of samples, epidemiological consequences, etc. Bearing these comments in mind it is of interest to examine the variations in residues between samples collected in different geographical areas, or at different times in the same geographical area.

GEOGRAPHICAL VARIATION

Europe.—The residues of organochlorine insecticides in the adipose tissue of members of the general population of Great Britain have been more intensively studied than those of other European countries. One group of workers has studied the population of south-east England (more specifically, London and the county of Kent) (6, 12, 31–33). Another group of workers analysed samples from various parts of Britain (14, 15), and one survey of samples collected in Somerset, in south-west England, has also been published (34). The mean values for the different geographical regions are very similar, a conclusion which is not particularly surprising in view of the small area and high population density of Britain. Analytically significant amounts of pp′-DDT + pp′-DDE materials (average about 2 to 3 ppm), HEOD (average about 0.2 to 0.25 ppm), and BHC isomers have been found. Traces of heptachlor epoxide were reported in one survey (14) but not in another (6). Analytically significant residues of endrin have not been reported, and residues of op′-DDT, op′-DDE, and pp′-DDD were usually below the limit of detection (6).

Surveys of residues of DDT-derived materials have been made in Denmark (35), West Germany (36), Holland (37, 38), Belgium (39), France (40), East Germany (41), Poland (42), Czechoslovakia (27), Hungary

FIG. 2. Concentrations (in ppm) of DDT-type compound and HEOD in adipose tissue in Europe; concentrations of HEOD in italics.

(26), and Italy (43, 44). There is a general tendency (see Fig. 2) for the residues in east and south Europe to be greater than those in west and north Europe. Studies of residues of HEOD have been made in Denmark (35), Holland (38), and Italy (43, 44) as well as in Britain; the residues in Italy are 2 to 3 times higher than in the other three countries. Results for BHC isomers have been reported (in addition to those for Britain) in Holland (38), Belgium (39), East Germany (41), and Italy (43, 44). With regard to heptachlor epoxide, residues [apart from the traces found in one survey in Britain (14)] have only been found in Italy (43, 44). However, the analytical procedure used in several of the surveys is such that the absence of results for a particular compound cannot be regarded as indicating that analytically significant amounts of that compound are absent.

North America.—The first survey of organochlorine insecticides in

human adipose tissue was that of Laug, Kunze & Prickett (10); the specimens were collected in California and analysed for DDT-type material by the Schechter-Haller colorimetric method. Residues of this class of compounds have been determined in specimens collected from different areas in North America during the past 18 years. There was a radical change in the analytical technique from about 1962 (see below) and this complicates the interpretation of the results with regard to distribution in space and time. The Schechter-Haller colorimetric procedure was used for the analysis of specimens of adipose tissue collected in Washington and Georgia (11), Alaska (45), Canada (46). Dale & Quinby (47) analysed samples from three states (Washington, Arizona, and Kentucky) and Quinby et al. (48) collected samples from four states (Washington, Arizona, Kentucky, and Georgia). The residues of DDT-type material were fairly similar in the four states, except that the concentration of DDE in Phoenix, Arizona, was about twice those found in the other samples. Dale & Quinby (47) also analysed their samples by gas-liquid chromatography using a micro-coulometric detector. This method of analysis was also used for samples collected in New Orleans (18), and Chicago (49). Other surveys have used the electron capture detector, the samples being collected from people living in Florida (8, 16, 20), from four geographical areas (13), and from Toronto, Canada (50). There are quite large variations in the mean concentration of total DDT-type material in these surveys, the residues in Alaska, Canada, and the north east of the United States being about one third to one half of those in other areas (see Fig. 3). Residues of HEOD, BHC isomers, and heptachlor epoxide have been reported in those surveys using suitable analytical methods. Once again quite large variations are found in the mean concentrations and some of the values found for HEOD are given in Figure 2. Whether the differences in the mean residues are indications of real geographical variations cannot be stated categorically in view of the possible effects of the variables discussed above and the variations in the analytical techniques used in different surveys.

Other countries.—Two surveys of DDT-derived materials have been made in Israel (5, 19). Studies of DDT and the other organochlorine insecticide residues have been made in Australia (51, 52), Hawaii (9), India (4), and New Zealand (53). A comparison of the results of these surveys with those in Europe and North America indicates that the residues of DDT-derived materials are higher in Delhi, India, and in Israel, than in the other countries. On the other hand, the concentration of HEOD in samples from Delhi and Hawaii was considerably smaller than those found in most countries.

Changes of Residues with Time

Surveys at different times have been made in Canada, Israel, Great Britain, and the United States. The average concentration of DDT-type material in human adipose in Canada in 1966 (50) was similar to that found in

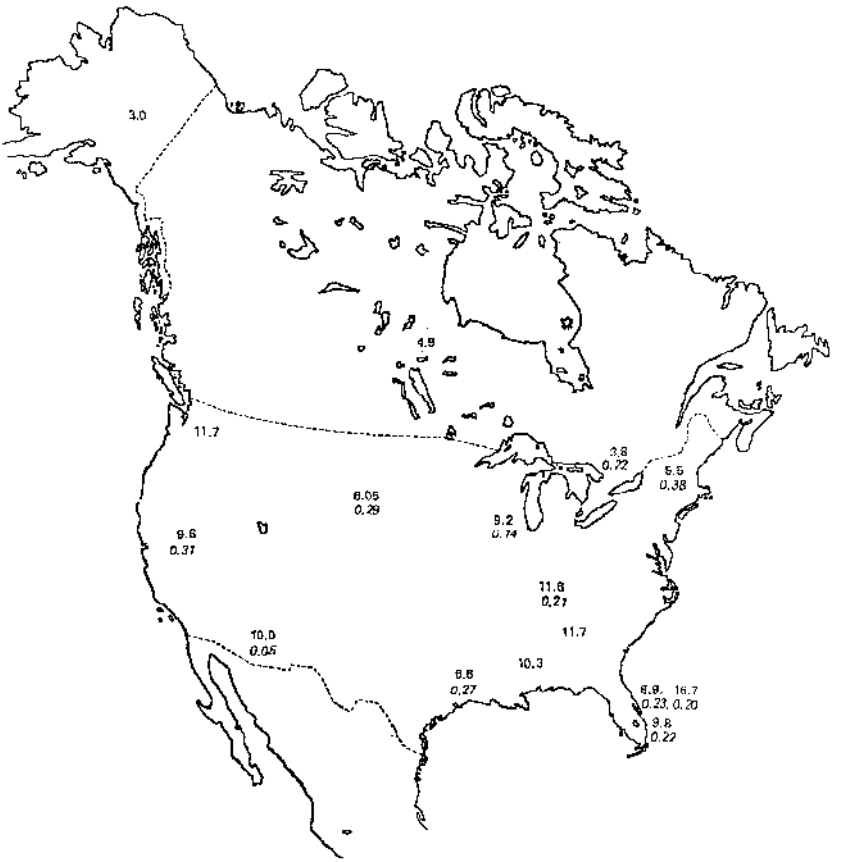

FIG. 3. Concentrations (in ppm) of DDT-type compound and HEOD in adipose tissue in North America; concentrations of HEOD in italics.

1949–50 (46); in Israel no significant changes occurred between 1963–64 (5) and 1965–66 (19). In Great Britain the average residues of organochlorine insecticides in adipose tissue found in several surveys between 1961 and 1965–66 were very similar. However, Abbott et al. (15) concluded that the concentrations of BHC, dieldrin, and DDT were lower in samples collected between 1965–1967 than in samples collected in 1963–1964; and Robinson & Roberts (33) concluded that the concentrations of HEOD in samples collected in 1968 were significantly lower than those found in earlier surveys in south-east England. In the United States Quinby et al. (48) expressed the opinion that there had been no change, between 1950 and 1961–62, in the concentration of either DDT or DDE that could not be attributed to improved analytical methods. Hoffman et al. (49) concluded that there had

been no general progression of storage of DDT in the general population since 1951, and that the concentrations in samples collected in Chicago were lower for BHC and DDT-type materials in 1964–66 than in 1962–63. Hoffman (54) stated that there appeared to have been no increase in the concentration of DDT + DDE in human fat since 1951 in the United States; Quaife, Winbush & Fitzhugh (55) made a critical review of the results of surveys of DDT-type material in human fat in the United States. They drew attention to some of the limitations of the published results and concluded that, subject to these limitations, the mean values for DDT + DDE had not been constant since 1950, but may have been since 1958. The limitations to which Quaife et al. refer are based upon their doubts whether the samples in the surveys they considered (covering the period 1960–1964) adequately represented the general population of the United States in relation to geographical origin of the samples, age, sex, state of health, diet, and environmental exposure to pesticides. The above discussion of these and other factors indicates that there are good grounds for reservations on the design of the sampling procedures used in some surveys in North America. The importance of properly designed sampling schemes has been stressed (3, 6), as biased samples may otherwise be obtained. It may be necessary to use stratified sampling schemes, the stratification being based upon those factors (other than adventitious exposure) which are found to affect residues in tissues in a significant manner in a particular country or geographical area.

Residues in Other Body Tissues and Fluids

The majority of surveys of residues in the general population have been based on samples of adipose tissue. Systematic surveys of residues in human blood have been made in Great Britain (31–33). Residues of organochlorine insecticides in other tissues have not been studied intensively but some results are available for liver (8, 9, 20, 38, 39), brain (8, 9, 20, 38), kidney (9, 20, 39), gonads (9, 20), and spleen (9, 39). Samples of human milk have been analysed by a number of workers. Average concentrations of total DDT-type material in the United States range from 0.06 to 0.19 ppm (10, 56, 57); in Britain the average found in one investigation was 0.13 ppm (14). The similarity of the average concentrations is surprising as the average concentration of DDT-type material in adipose tissue in Britain is only about one third of that in the United States. Residues of BHC isomers and HEOD in human milk have also been determined in Britain (14); the average concentration of HEOD was 0.006 ppm (compared with 0.003 ppm in cows' milk), in the United States the average concentration was also 0.006 ppm (56), [0.003 ppm in cows milk (58)]. The relatively high concentrations of these compounds in human milk in Britain and the United States as compared with cows' milk is very striking. The residues of these compounds in human milk are also of interest in regard to the tendency of the concentration of these compounds in the adipose tissue of infants to decline during the first three months after birth (15), and it appears that the rate of deposition of adi-

pose tissue may be sufficient to counteract the rate of ingestion of these compounds. One may also speculate that either the efficiency of absorption of organochlorine insecticides is lower in infants than in adults, or that they are more rapidly eliminated by infants. No firm conclusions can be drawn, however, from the presently available data as we do not know the numbers of breast-fed and bottle-fed infants included in the surveys.

Analytical Methods

Detailed reviews of analytical methods for pesticides have been given by Beynon & Elgar (59) and Williams & Cook (60). Frazer (61) also reviewed analytical methodology. Manuals of analytical methods have been published by Burchfield, Johnson & Storrs (62) and by Barry, Handley & Johnson (63).

A detailed review of recent developments in the analytical methods for the organochlorine insecticides is not considered necessary, but there are certain aspects of these methods that warrant discussion. The analytical procedures used in the majority of the recent surveys of the concentrations of organochlorine insecticides in human tissues involve the following steps: extraction, clean-up, gas-liquid chromatography, quantitative detection.

Extraction.—In the ideal extraction procedure the residues of organochlorine insecticides present in the sample of human tissues are transferred completely into a suitable solvent. The residues present in the tissue may be present in several different states such as solution in lipids, protein-solute complexes, or both. Further, these physico-chemical states may be present in a variety of organelles: cell walls, mitochondria, etc. Therefore, in order to ensure adequate contact between the solvent and the pesticide, it is necessary that all cellular structures should be broken physically or chemically, e.g., by grinding with sand, by alkaline hydrolysis, or some other suitable procedure. Alkaline hydrolysis, of course, may be used only for the extraction of compounds that are stable to alkali. Exhaustive extraction using a Soxhlet apparatus may be used as the final step. The measurement of the efficiency of the extraction procedure is often unsatisfactory. The standard method is to fortify a sample of a control tissue (if such is available), or an extract therefrom, with known amounts of the compound under investigation and to calculate the recovery by comparing the amount found by the analytical method with the amount added. This type of procedure, as has been frequently pointed out in the case of crops, does not necessarily give an accurate measure of extraction efficiency (64–68). In most circumstances the only absolute method of measuring extraction efficiency is to use an isotopically labelled compound (65). Such a study of the extraction efficiency in the case of residues of HEOD in animal tissues has been made by Greichus et al. (69). Another approach is the chemical destruction of the normal tissue constituents, lipids, proteins, etc., so that a homogeneous hydrolysate is obtained. It is then possible to measure extraction efficiency since the problem has been reduced to the simple one of measuring the partition coefficient

of the compound between the hydrolysate and a suitable solvent. Alkaline hydrolysis has been used in the estimation of HEOD, and DDT-type compounds (in the form of the ethylene derivatives) in adipose tissue (6) and blood (70, 71). Both the isotopic method and the hydrolytic method indicated that solvent extraction procedures can efficiently extract organochlorine insecticides from tissues. It must be stressed that both these methods of measuring extraction efficiency must be used with care if misleading results are to be avoided. For example, it was found that HEOD incorporated in liver tissue by normal biological process is not stable to alkali (72); reductive dechlorination occurs to give two isomeric pentachloro compounds. A procedure for proteinaceous tissue in which the latter is first extracted with a solvent and the extract is then hydrolysed with alkali has been described (73). This procedure may overcome the reductive dechlorination problem found with liver, but the efficiency of the extraction cannot be measured directly. Some extraction procedures use hexane, a nonpolar solvent (74, 75). The efficiency of these procedures requires close examination, preferably by experiments with isotopically labelled molecules. It is noteworthy, for example, that a comparison of the concentrations of HEOD in human blood found by two extraction procedures, one using a polar solvent, the other hexane, gave significantly different results (76). The literature on the determination of pesticide residues in foodstuffs is large and growing, and the comparison of various extraction procedures for residues in whole prepared meals (77) is relevant to this discussion of the extraction of such residues from human tissues.

Clean-up.—No outstanding developments in clean-up procedures have been made recently. The use of charcoal columns has been studied and it is noteworthy that the order of elution of DDT-type compounds relative to HEOD, endrin, and heptachlor epoxide, is the opposite of that found with silica gel or alumina columns (78). Morley (79) has reviewed the information on adsorbents and their use in column chromatography. Alkaline hydrolysis, which was referred to above, is a clean-up as well as an extraction procedure. The only surveys of residues in human tissues that have not involved a clean-up step prior to gas-liquid chromatography are those of a group of investigators in Florida (8, 20, 75). These workers stated (75) that interference by co-extracted materials was reduced to a minimum by using an electron capture detector at its maximum sensitivity. As the electron capture detector is not specific for organochlorine compounds (80) the validity of the concentrations reported by these workers is dependent upon the efficiency of separation of the gas-liquid chromatographic step in the analysis.

Gas-liquid chromatography.—Since the application in 1960 of the microcoulometric and electron capture detectors for the determination of organochlorine insecticides (81–84), gas-liquid chromatography in combination with these detectors has become the method of choice in residue analysis. The microcoulometric detector provides one of the most dependable analyti-

cal systems, and has been used for the determination of organochlorine insecticides in adipose tissue in a number of surveys (7, 18, 47, 49). The electron capture detector, with its increased sensitivity of response, has been used more widely, but the particular combination of this detector with gas-liquid chromatography has created considerable misinformation and misunderstanding (85). This misunderstanding is the result of the nonspecific response of the electron capture detector combined with an apparent failure to appreciate the basis of gas chromatography. Gas chromatography is essentially a method of separating the components of a mixture and the use of gas chromatographic criteria as indices of identity requires considerable care. The mere similarity of the retention volume of a component in a tissue extract with that of a known organochlorine insecticide is not sufficient evidence that the component is chemically identical with that insecticide. This point has been emphasized by a number of investigators, and a convenient review of the general topic of gas chromatographic identification has been made by Leathard & Shurlock (86). Although the limitations of gas chromatography as an identification technique are now more widely realised, the attempts to overcome these limitations have so far been mainly of an empirical nature. Methods of qualitative confirmation of identity are considered below, except those involving gas chromatographic procedures which are discussed in this section. A number of compounds have been evaluated as stationary phases for the separation of organochlorine insecticides (82, 86–92). No single stationary phase, under the conditions investigated so far, will separate all the known organochlorine insecticides and related compounds. A common method used to overcome this difficulty is to use two or more stationary phases (84, 87, 92–94). The use of two or more columns to identify a compound involves an assumption, namely, that the retention volumes obtained with different stationary phases are independent. Unless this condition is fulfilled the total information regarding identity provided by the retention times obtained with two stationary phases is not an additive function of the information provided by the retention volumes on the two stationary phases. The independence of retention volumes appears to be unlikely on a priori grounds, and it has also been shown experimentally (95) that retention volumes obtained with one stationary phase are correlated with those on a second stationary phase. These observations form the basis of an attempt by Robinson et al. (95) to give an operational definition of chemical identity suitable for use in microanalysis. This definition of identity is based on class algebra and it has affinities with concepts developed by other workers (96–99). It has been pointed out (100) that, in most papers on the determination of organochlorine insecticides, insufficient details on the performance of the gas chromatographic column (e.g., number of theoretical plates, separation factors) are given, and that the descriptions of chromatographic conditions should be standardised by giving such details.

Detection systems.—A variety of detection systems has been used in gas chromatography and a review of them has been published by Westlake &

Gunther (101). Only two of these systems, the microcoulometric and electron capture detectors, have been used in the determination of organochlorine insecticides in human tissues. No direct comparison of the results obtained by the two detection systems in the analysis of adipose tissue, etc., has been published. Comparisons of the concentrations of DDT-derived materials, determined by the Schechter-Haller colorimetric procedure and gas-liquid chromatography with microcoulometric detection, have been made (4, 40, 47). The results of these comparisons are inconsistent with one another. This may have arisen partly from the ease of thermal degradation of pp'-DDT unless the gas chromatographic conditions are strictly controlled (102), and partly from the relative lack of specificity of the colorimetric procedure. A comparison of the residues of DDT-derived materials in adipose tissue, determined by thin layer chromatography and gas chromatography, was made by Engst et al. (41). The correlation coefficient for the results obtained by the two methods ($r = 0.92$, $P < 0.001$) indicates that the two methods give highly concordant results. Since retention volumes and R_f values appear to be independent parameters (95) it may be concluded that both methods give valid estimates of the concentration of DDT-type compounds. A number of the other detection systems appear to be suitable for the routine analysis of tissues. These include the leak detector (103, 104), flame emission detectors (105, 106), and thermionic detectors (107, 108). Electron capture detectors in which ^{63}Ni (109) or photoionisation (110–112) is the source of electrons have been described. There is growing interest in mass spectrometric detection systems, used in conjunction with gas chromatography by means of a suitable pressure reduction system (113, 114).

Confirmatory techniques.—The increasing appreciation of the deficiencies of gas chromatographic systems and the detection systems used in the last few years has resulted in the investigation of various qualitative confirmatory techniques. Thin layer chromatography has been used most frequently for this purpose in the case of residues in human adipose tissue. Chemical reactions (115, 116) have been used but this technique has not been applied widely to human tissues. Infrared absorption spectrophotometry is a powerful tool for establishing chemical identity, but its use is restricted both by its relative lack of sensitivity and by difficulties in isolating a sufficiently pure specimen which will give a reliable spectrum. Mass spectrometry is another powerful tool. Perhaps the most intensive investigation of the identity of a component in human adipose tissue, suspected to be an organochlorine insecticide, is the study in this laboratory of the identity of the component which has been estimated as HEOD by gas chromatography. The confirmatory techniques included thin layer chromatography, chemical reactions, infrared and mass spectrometry (3). Samples that were collected and stored before the introduction of a particular insecticide have also been used in confirmatory studies (6, 11, 117). However, this particular form of proof of identity requires care: it has been found that the concen-

tration of HEOD or DDT-type compounds in these museum specimens were at or below the limit of detection of the analytical techniques used, and it has been concluded that this indicates that the components present in modern specimens are therefore pp'-DDT or HEOD as the case may be. This conclusion only follows if it can be shown that no other compounds that may simulate the behaviour of these compounds have been introduced into the human environment in the past 25 years or so; the chlorinated biphenyls, for example, may be an example of such compounds. A useful confirmatory technique, which has the advantages of simplicity and sensitivity, is the determination of partition p-values as described by Beroza & Bowman in a series of papers (118–123).

Limits of detection.—Not all the specimens analysed have been found to contain analytically significant amounts of all the organochlorine insecticides. For example, endrin is not found in samples of adipose tissue in Britain at the limit of detection of the analytical method used, <0.02 ppm (6). In many reports the term "limit of detection" appears to be used without proper definition, and this is another aspect of analytical methodology for organochlorine insecticides that should be standardised (100). The limit of detection of a given procedure may be defined as that amount of a compound which produces no significant response from the detection system. A significant response is either one which is greater than the noise level of the detector when an authentic control sample (containing none of the compound of interest) is used, or one which is greater at the time, absorption wavelength, etc., corresponding to the component, than the background noise adjacent to this time or absorption wavelength. This topic has been discussed by McWilliam (124), Young (125), Johnson & Stross (126, 127), Currie (128), and Jones (129).

Metabolism

Previous reviews of the metabolism of organochlorine insecticides have been given by Hayes (130) and Frazer (61); Brooks (131) and Korte (132) have discussed the metabolism of the cyclodiene insecticides (i.e., insecticidal compounds synthesised by the Diels-Alder reaction). The fate of organochlorine insecticides in man and other vertebrates may be considered, first, in relation to their biotransformation; and, second, by the complementary approach in which the dynamics of their uptake, distribution, storage, and elimination are considered.

Biotransformation.—Considerable attention has been given recently to the conversion of pp'-DDT to pp'-DDD. This biotransformation was first reported by Finley & Pillmore (133) in experiments with cottontails. Barker & Morrison (134) concluded that the presence of pp'-DDD in the tissues of mice that had been dosed with pp'-DDT was the result of post-mortem decomposition during storage of tissues, and that it did not occur in normal live tissues. These two groups of workers had used paper chromatography in their analysis of tissues. Datta et al. (135) detected pp'-DDD in

rat livers, but not in body fat or kidneys, by gas chromatography. Peterson & Robison (136) also reported the formation of pp′-DDD and suggested a sequence of successive dehydrochlorination and reduction reactions by which the side chain chlorine atoms in DDT could be removed. Miscus et al. used ^{14}C-DDT in experiments in which conversion of DDD was found to occur in bovine rumen fluid, and aqueous solutions of reduced porphyrins were also found to give this product (137). This latter finding is analogous to that of Castro (138), namely, the formation of pp′-DDD in the presence of ferrous deuteroporphyrin under anaerobic conditions. In contrast to the conclusions of Barker & Morrison, Mendel & Walton (139) considered that the normal flora of the gastro-intestinal tract of the rat was the major agent in the formation of pp′-DDD, and Braunberg & Beck (140) concluded that pp′-DDD in the faeces of rats fed pp′-DDT was largely the product of microbial rather than mammalian metabolism. Support for post-mortem breakdown as the source of pp′-DDD in tissues is given by the results of analyses of livers of birds that had been dosed with pp′-DDT (141, 142). Biotransformation in the urine of cattle was suggested by McCully and co-workers (143, 144). Ottoboni and her co-workers investigated two of the proposed mechanisms, namely, hepatic and bacterial metabolism, by determining the ratio of the concentrations of pp′-DDT and pp′-DDD in the liver and body fat of rats (145), and by the use of axenic rats (146). They concluded that the contribution of intestinal microflora to the formation of pp′-DDD was small compared with that of the hepatic mechanism. Bailey et al. (147) studied the metabolism of pp′-DDT and pp′-DDD in pigeons, and they also studied the metabolism of pp′-DDE and that of the dehydrochlorination production of pp′-DDE [2,2-bis(p-chloro-phenyl)1-chloroethylene] (148). The formation of polar metabolites of DDT, which are probably hydroxylated derivatives, has been reported by Morello (149) and Sanchez (150). A different type of reaction has been reported by Klein et al. (151), namely, the formation of pp′-DDT in rats fed op′-DDT.

The metabolism of several of the cyclodiene insecticides has been studied by a number of workers. Heath & Vandekar (152), using ^{36}Cl-HEOD, found that in rats about 90 per cent of the total dose was excreted as hydrophilic metabolite in the faeces, and about 10 per cent in the urine. The major metabolite in the faeces was neutral and somewhat more polar than HEOD. Also ^{36}Cl-ion was detected in the urine. This latter observation is consistent with the detection of a metabolite in rat urine (153) which has been independently identified by two groups of workers (154–158) as a pentachloro compound: 1,1,2,3a,7a-pentachloro-5,6-epoxy decahydro-2,4,7-methano-3H-cyclopenta (a) pentalen-3-one. The urine of aldrin dosed rats was reported to contain an isomeric metabolite, possibly differing from that formed by HEOD in the cis- configuration of the epoxide ring. However, the evidence for this isomer does not seem conclusive. It is of interest that the photoisomerisation product of HEOD (see below) has been found to give a metabolite which is identical with the keto-com-

pound found in the urine of rats fed HEOD (M. Baldwin & J. Robinson, unpublished work), Richardson et al. (157, 158) also isolated a metabolite from the faeces of rats fed HEOD and this was identified as a monohydroxyl derivative. Cueto & Hayes (159) reported the occurrence of two neutral, polar metabolites of HEOD together with HEOD itself in the urine of men with an occupational exposure to dieldrin. The occurrence of HEOD in human urine was also studied by Cueto & Biros (160), and Hayes & Curley (161), and it was suggested that the concentration of HEOD in urine could be used as an index of exposure to dieldrin. Unpublished work in this laboratory has not confirmed that this is a suitable index of exposure to aldrin and dieldrin. Korte and his co-workers at the University of Bonn have published a great deal of information on the metabolism of cyclodiene insecticides. A number of metabolites of aldrin and dieldrin have been detected in the tissues and excreta of rats but their identities were not established (162). The major metabolite of HEOD in the urine of rabbits was identified as trans-6,7-hydroxy-dihydro-aldrin, and two other metabolites were converted to this compound by hydrolysis (163). In rabbits given alpha-chlordane hydrophilic metabolites were found in the urine and faeces in the approximate ratio of 2:1. One of the urinary metabolites was identified as the chlorohydrin. The other was more polar and was presumed to be the corresponding diol (132). A hydrophilic metabolite of heptachlor has been identified as 1,2,3,4,8,8-hexachloro-5-*exo*-hydroxy-6,7-epoxy-1,4,4a,7a,5,6-hexahydro-1,4-*endo*-methylene-indene (164). In a study of rats dosed with ^{14}C-endrin, Klein et al. (165) reported finding two metabolites in the tissues. The major metabolite was characterised chromatographically as the isomeric ketone, 1,8-*exo*-9,10,11,11-hexachloropentacyclo (6,12,1,1,^{3,6},0^{2,7},0^{4,10}) dodecan-5-one, but investigations in this laboratory indicate that this identification is incorrect.

Mechanisms of biotransformation reactions.—The main biotransformation reactions may be classified formally as follows: hydroxylation, reductive dechlorination, dehydrochlorination, and transformation of the oxirane ring to a diol. The mechanisms of these various reactions (which may be accompanied in some cases by other reactions) are only slightly understood. Dehydrochlorination, for example, is attributed to a dehydrochlorinase enzyme, but this is almost tautological as regards understanding the reaction mechanism. The conversion of pp'-DDT to pp'-DDD by *Aerobacter aerogenes* has been shown to occur by direct reduction rather than by dehydrochlorination followed by reduction (166). The hydroxylation reactions probably involve enzymes of the mixed function oxidase type (167) in which one atom of oxygen is incorporated into the substrate molecule. The precise mechanism of the oxidation reactions is not known, but one possible mode of reaction is formally similar to an electronphilic substitution reaction in which the electrophile is either OH^+ (168) or some entity which acts as if it were this ion. The formation of the two metabolites of HEOD may be explained on this basis (169).

Pharmacokinetics.—The study of the dynamics of the uptake, distribution, and elimination of a compound is complementary to the study of its biotransformation products. A large amount of empirical information is available on the various aspects of the pharmacokinetics of the organochlorine insecticides, and the information up to about 1967 has been summarised by Hayes (130), Robinson & Roberts (170), and Robinson (3, 171). A great deal of this information was obtained in toxicity studies which were often not designed with a view to obtaining pharmacokinetic data. Consequently, there were a number of gaps in the earlier results, but Robinson (171) proposed four postulates or axioms to summarise the dynamics of these compounds during chronic exposure. Systematic investigations of the pharmacokinetics of HEOD in man, rats, dogs, and birds (169, 172–175) have given results which are consistent with the postulates, and the results of a number of ad hoc studies with DDT and dieldrin are also consistent with them. Hunter & Robinson (172, 173) carried out a two year study of HEOD with human volunteers in which there was a control group and three treated groups, and it was shown that the concentrations of HEOD in blood and adipose tissue were related to the daily intake. On the basis of all the results obtained over the two year period, relationships were derived between the intake and the concentration in the tissues. These relationships may be used to estimate the mean total equivalent oral exposure to HEOD of the general population if reliable estimates of the mean concentrations in blood or adipose tissue of members of the general population are available. Similar relationships between intake and the concentrations in tissues have also been derived for rats and dogs (174). Richardson et al. (176) found significant relationships between the concentration of HEOD in the blood and the concentrations in five other tissues, but nonsignificant relationships in the case of the spleen and pancreas. However, the number of animals on this trial was small and too much significance should not, perhaps, be attributed to the lack of correlation between the concentrations in these two tissues and that in the blood. The functional relationships between the concentration of HEOD in the blood and that in other tissues have been studied for HEOD in man (173), and rats and dogs (29, 174) and for DDT-type compounds in cattle (177). The asymptotic form of the relationship between the concentration of HEOD in the blood and the time of continuing exposure has been established unequivocably in man (173), and the results of two year studies with rats and dogs (174) and a shorter term study with dogs (29), are consistent with this concept, as are those obtained with pigeons (175). The mechanism of this tendency to an upper limit of storage for a given intake, which corresponds to a balance between the intake, storage, and elimination of HEOD, has been elucidated by a detailed study of the elimination of HEOD from the tissues of rats (169) in which the rates of elimination of HEOD from blood, adipose tissue, liver, and brain were determined. Rumsey et al. (178) found a significant decline in the concentration of total DDT-type compounds in the tissues of cattle when exposure

was terminated, but too few results were obtained to determine the relationship between the rate of decline and concentration. The rate of transfer of DDT from the blood after direct injection into the jugular vein of cows was studied by Witt et al. (179). An equilibrium concentration was reached in about 24 hours, but these workers were unable to determine whether the change in concentration in the blood could be represented by a classical two compartment process (see below).

A valuable technique for studying the distribution of organochlorine insecticides in the body tissues in a qualitative fashion is the use of autoradiography. Backstrom et al. (180) studied the distribution of both ^{14}C-DDT and ^{14}C-dieldrin in pregnant mice by whole body autoradiography, and Woolley & Runnels (181) used this technique to determine the distribution of DDT in the brain and spinal cord of the rat. This study is complemented by Schwabe's investigation of the distribution of ^{14}C-DDT in eight different parts of the brains of cats; a scintillation method was used in these investigations (182). The placental transfer demonstrated by Backstrom et al. has, of course, been demonstrated by other techniques (22–25, 183).

Mathematical models.—It has been suggested that the dynamics of organochlorine pesticides in warm-blooded animals could be explained by regarding an animal as consisting of a number of compartments in which the compounds are stored. Robinson (184, 185) tentatively discussed the behaviour of HEOD on the basis of the mamillary-type of compartmental model and Potter & Porter (186) suggested a nonmamillary compartmental model. More explicit discussions of the dynamics of HEOD in terms of a compartmental model, in man and animals, were given by Robinson (3, 171), and the results of studies of rats and dogs (174), and man (173) have been shown to be consistent with the mamillary-type model. In a detailed discussion of the pharmacokinetics of HEOD in the rat (169) it was concluded that the results were consistent with a two compartment model, the circulating blood, liver, and brain being contained in the central compartment, and the depot fat comprising the peripheral (storage) compartment. It must be stressed that this two compartment model represents the behaviour of HEOD in the case of chronic exposure. In short-term acute exposures it is probable that a more complex model, consisting of three or more compartments, may be necessary. The results of acute intoxication studies of DDT in the rat (187) give some indication that this more complex model may be necessary in such circumstances.

The detailed metabolite pathway(s) have not been discovered for any of the organochlorine insecticides and considerably more work is required in this field. A better understanding of the mechanisms of biotransformation of this particular class of xenobiotics may enable molecules to be synthesised that do not have some of the properties of these compounds (e.g., relative stability in vertebrate organism) which are currently considered undesirable by many toxicologists and ecologists. The dynamics of the behaviour of HEOD in vertebrates has been rationalised on the basis of an appropriate

mathematical model, but there is an unfortunate dearth of systematic studies of the other organochlorine insecticides.

Photochemical Reactions of Organochlorine Insecticides

The degradation of DDT was investigated by Fleck (188), and Roburn also studied other insecticides (189). The structure of the photolysis product of HEOD has been established by chemical and physical methods (190–192), and the proposed structure is consistent with the formation of this compound by the epoxidation of the photoisomer of aldrin (193). An alternative structure for the photoisomer of HEOD, proposed by Harrison et al. (194) is inconsistent with this synthesis. Rosen (195) investigated the photolysis of aldrin, and Henderson & Crosby (196, 197) have also studied the photolysis of dieldrin in organic solvents and in water. Li & Bradley (198) showed that the photolysis products of methoxychlor were chemically analogous to those of pp′-DDT. The insecticidal and toxicological properties of the photoisomer of HEOD have been investigated (191, 199) and residues of this compound in certain foodstuffs and in human adipose tissue have been determined (190, 200); most of the residues are below the limits of detection.

In spite of the relative stability of the organochlorine insecticides they are, nevertheless, susceptible to physiochemical and biological degradation. It is becoming increasingly apparent that the chemical nature of terminal residues of these compounds is very complex, and it is probable that too little attention has been given to this problem. Some re-allocation of analytical effort, which is currently concentrated upon monitoring residues of the organochlorine insecticides per se, is required if a balanced picture of the total incidence of residues is to be attained.

Microsomal Enzyme Induction

The ability of a wide variety of compounds, including organochlorine insecticides, to increase the activity of drug metabolising enzymes in the liver has been studied intensively in recent years (201). The initial observation of this property of organochlorine insecticides arose from an incidental observation on the activities of drugs in animals housed in rooms that had been treated with organochlorine insecticides. Hart & Fouts showed subsequently that both DDT and chlordane stimulate microsomal drug metabolising activity (202, 203). Interactions of this type have been found between the various organochlorine insecticides and a wide variety of drugs in several animal species (204–207).

Increased metabolism of steroids.—Interactions between organochlorine insecticides and steroids have been demonstrated (208, 209). Conney and his co-workers have studied the enhancement of the metabolism of estradiol-17β, testosterone, progesterone, and desoxycorticosterone (210–212). The dosages used in these trials were large, e.g., 25 mg DDT/kg body weight, intraperitoneal injections twice daily for 10 days (210). Peakall (213) stud-

ied the increased metabolism of testosterone and progesterone by the liver microsomal enzymes of pigeons dosed with DDT or dieldrin. Risebrough et al. (214) extended this study to include pp'-DDE, technical DDT, and Aroclor 1262 (a polychlorinated biphenyl): intramuscular injections of 40 mg DDE or DDT produced significant increases in the hepatic microsomal metabolism of estradiol-17β.

Increased metabolism of organochlorine insecticides by drugs.—The metabolism of organochlorine insecticides is increased in animals treated with various drugs, such as phenobarbital, aminopyrine, chlorpromazine, phenylbutazone, tolbatamide, etc. (215–220). Street (220) also correlated the reduction of storage of dieldrin in the body fat of rats, consequent upon treatment with various drugs, with increases in the excretion of urinary ascorbic acid, and the activity of aniline oxidase in liver homogenates, and with reductions in sleeping times.

Interactions between organochlorine insecticides.—Street (221) showed that the storage of HEOD in the body fat of rats was reduced by simultaneously feeding them with DDT. Morello (149) demonstrated that DDT enhanced its own rate of metabolism by hepatic microsomal enzymes, and a variety of interactions of this type have been found (221, 223, 227). An interaction leading to enhancement of storage has been reported by Deichmann et al. (228); the concentrations of pp'-DDT + pp'-DDE in the body fat and blood of dogs was increased by feeding them with DDT + aldrin.

Hepatic Microsomal Enzyme Induction in Man

The estimated average daily intakes of DDT-type compounds in the U.S.A. and U.K. are about 0.001 mg/kg body weight (58, 228–231), and in these two countries the corresponding intake of HEOD is in the range 0.0001–0.0002 mg/kg body weight (58, 229–231). The hepatic microsomal enzyme induction effects that may arise from these exposures can only be assessed, at present, by extrapolation from animal experiments. The results of many investigations are obviously irrelevant to this assessment as the dosages employed were relatively huge, and intraperitoneal injection was often used. The investigations of Gerboth & Schwabe (232), Schwabe & Wendling (233), Kinoshita et al. (234), Datta et al. (235), Gillett & Chan (226), and Street (220) involved treatments which were rather more realistic in terms of the exposure of the general population. Dietary intakes which produced no detectable changes in rats have been determined. These are of the order of 2 ppm for DDT, equivalent to about 0.2 mg DDT/kg body weight; and between 1 to 5 ppm for aldrin, dieldrin, heptachlor, and heptachlor epoxide, equivalent to about 0.1–0.5 mg/kg body weight. In some cases (232, 233, 235) the concentrations of DDT-type compounds in the body fat of the rats, in which increased microsomal activity had been found, were determined. These concentrations were of the order of 10 to 20 ppm, and it has been suggested that increased metabolism of drugs in the general population may be occurring as a result of the residues in man's tissues.

There is an ambiguity of the extrapolation of these animal trials to man, different conclusions may be drawn by comparing exposures (in terms of mg of insecticide/kg body weight) of rats and man on the one hand, and by comparing residues in adipose tissue on the other. No categorical conclusions can be drawn at present, and studies with human volunteers or men with an occupational exposure to organochlorine insecticides are required. In these studies suitable parameters of hepatic microsomal enzyme activity, e.g., excretion of glucaric acid or corticosteroids in the urine, should be determined, and any departures from the normal population values correlated with a suitable index of exposure to the insecticides. In the case of volunteers who had ingested up to 230 μg HEOD per day (0.003 mg/kg body weight per day), and whose body burden, as measured by the concentration of HEOD in adipose tissue or whole blood, was about 10 times that of the average person in the U.K. or U.S.A., the concentration of pp'-DDE in the whole blood did not decrease. It was tentatively concluded that the rate of metabolism of pp'-DDE in man was not increased by a ten-fold change in the body burden of HEOD (3).

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