Organochlorine compounds in man and his environment

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Organochlorine compounds are one of the many groups of chemicals that have come under the scrutiny of pollution conscious ecologists and conservationists. These compounds are certainly widely distributed throughout the biosphere, but detailed studies of their concentrations are fraught with analytical difficulties, which are not always appreciated. The problem of identifying compounds at the submicro level is particularly pertinent.

Many organic compounds containing chlorine are produced by the chemical and pharmaceutical industries. Apart from pharmaceuticals which are produced in relatively small amounts, the majority of industrial organochlorine compounds are aliphatic or aromatic chlorinated hydrocarbons. These are used as solvents, *e.g.* carbon tetrachloride, trichloroethylene *etc.*, flame retardant agents, plasticizer extenders, extreme pressure additives, dielectric fluids and insulators for electrical conductors, *e.g.* chlorinated biphenyls and naphthalenes. Their use is based, apart from their cheapness, on their general chemical inertness. For example, the chlorinated biphenyls are used as dielectric media in transformers since they meet the requirements of fire resistance, high dielectric strength and relatively high dielectric constant, high resistivity and very low power factor.

The large variety of synthetic organochlorine compounds manufactured and used in industry makes a sharp contrast with the scarcity in nature of organic compounds containing covalently bonded chlorine. Of these which do exist, the majority are secondary metabolites of fungi—a few dozen of these are known, *e.g.* caldariomycin, griseofulvin, drosophilin *etc.* A chloroperoxidase, which catalyses the chlorination of β-dicarbonyls, tyrosine, and a number of other compounds, by chloride ion in the presence of hydrogen peroxide, has been isolated in crystalline form from the mycelium of the mould *Caldariomyces fumago* (the source of caldariomycin). Preliminary kinetic studies[1] suggest that the first step in the reaction is the oxidation of the chloride ion to a cation or free radical, the overall reaction being:

$$\text{>CH} + H_2O_2 + Cl^- + H^+ \xrightarrow{\text{chloroperoxidase}} \text{>C} - Cl + 2H_2O$$

But such enzymic chlorination in nature is comparatively rare; and it may be postulated that the relative biochemical stability of the C–Cl bond in synthetic compounds may be related, at least in part, to the scarcity of natural organochlorine compounds.

Environmental distribution

Many of the industrial chlorinated hydrocarbons are used in enclosed systems, and so, apart from accidents or waste disposal, they are not released into the general environment. An exception to this may be the chlorinated paraffins used as dry cleaning solvents, which are relatively volatile. Nevertheless, in recent years, evidence has been obtained of the widespread occurrence in the environment of many compounds used chiefly in closed systems, *e.g.* the chlorinated biphenyls.

Apart from these, there is one group of organochlorine compounds which is used predominantly in the open environment—the organochlorine insecticides. They differ from most other organochlorine compounds not only in regard to their mode of use and the relatively small quantities produced each year, but also because they have a high biological activity—this is the reason for their use as insecticides. Their widespread distribution in the environment, relative stability and biological activity (which is not confined to insects despite their name) have caused growing concern about possible hazards to man and other organisms. In the past the problem has been aggravated by some carelessness in waste disposal, but episodes of this type have declined in recent years. Indeed, the investigation of the contamination of the environment by organochlorine insecticides, and of their impact (direct or indirect) on various types of organisms, including man, may be regarded as a paradigm of the study of the pollution of the environment by chemicals.

The various types of organochlorine compounds are illustrated in *Scheme 1*. It includes insecticides classified into two main types—chlorinated aralkanes and chlorinated cycloalkanes; chlorinated phenoxyacetic acids which are produced in relatively large amounts as herbicides; and fungicides such as hexachlorobenzene and pentachlorophenol. Chlorinated aromatics, which are produced for industrial use rather than as biocides, include chlorinated naphthalenes and biphenyls: there are over 100 isomers of the latter containing between two and 10 chloro-substituents. A number of compounds, which occur as trace impurities, are extremely toxic, *e.g.* chlorinated benzodioxanes and benzofuranes.

Some of these compounds are associated with deleterious effects on wild life, particularly predators at the ends of food chains. The literature on this topic is large and various reviews have been published, particularly on the organochlorine insecticides.[2–4] Certain aspects which are of particular interest to physical scientists are summarized here.

There have been many investigations of the residues of DDT-type compounds, HEOD *etc.*, in man and his diet,[5] and in a large variety of environmental samples such as the rainwater, Antarctic snow,

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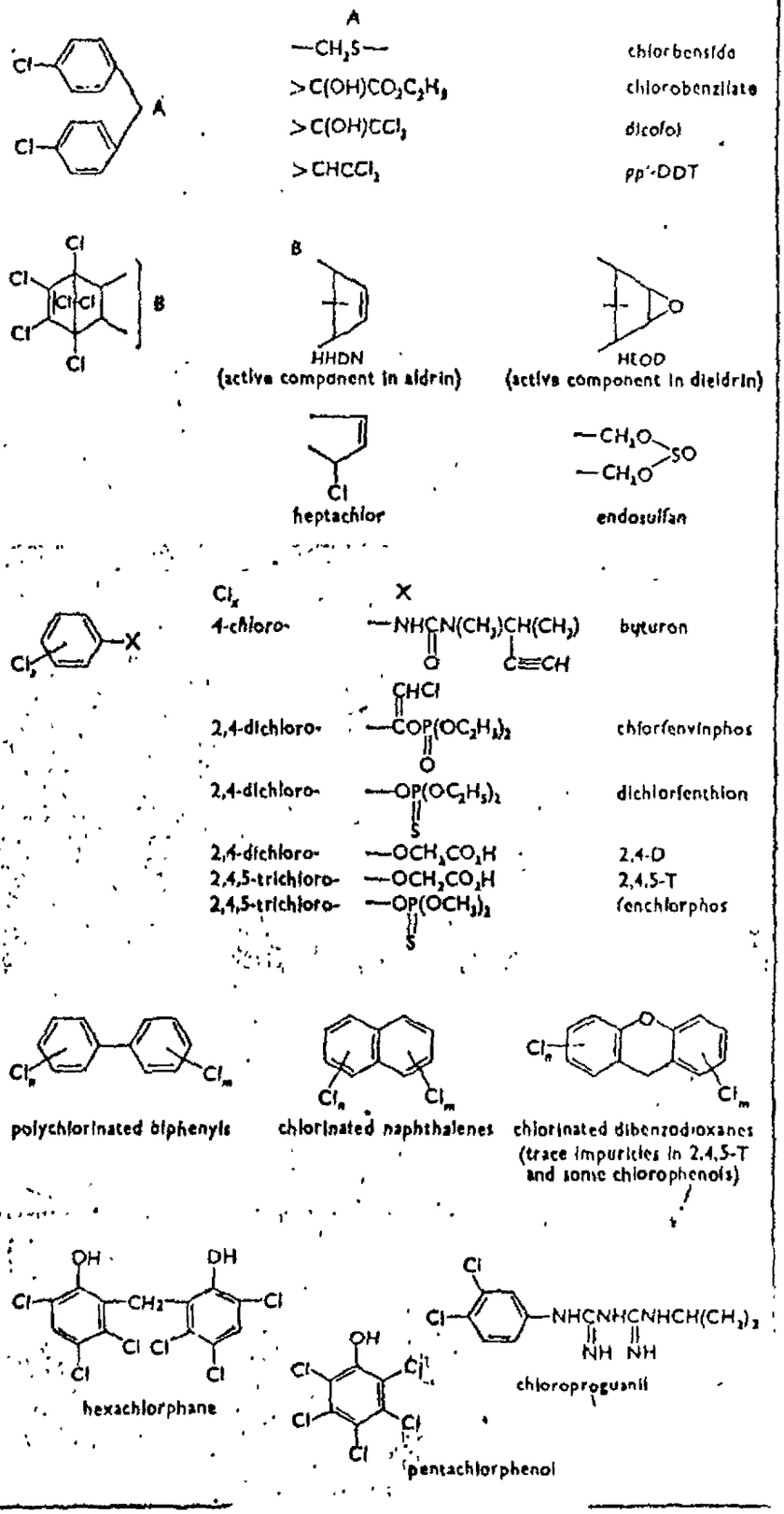
air, fish, tissues and eggs of birds etc. The results are generally interpreted as indicating the ubiquity not only of these compounds but also of polychlorinated biphenyls. It is probable that other organochlorine compounds will also be found as analytical techniques are developed. The widespread occurrence of these compounds can be attributed largely to their relative stability, both in the inorganic components of the environment, *i.e.* the atmosphere, hydrosphere, and upper part of the lithosphere, and in the various living organisms. The chemical and biochemical aspects of organochlorine compounds in the environment are discussed later. But first the analytical methodology used in relation to the study of the occurrence, distribution and persistence of these compounds in man and his environment are described.

Analysis

During the last 10 years analytical methods have been developed that are capable of measuring concentrations of organochlorine compounds over a range from about 10^{-5} to 10^{2} ppm. These methods are based on gas-liquid chromatography in combination with a detector of the electron-capture type. As with all analytical methods, the accuracy and reproducibility of the procedures are very important, but there are particular difficulties associated with analysing pesticide residues. The accuracy of the results, *i.e.* the concordance between the true values and the analytical results, are dependent on a number of factors corresponding to the various steps in the analytical method. First, the compound of interest must be completely extracted from the sample. Secondly, there must be no loss of this compound during the procedures by which the relative concentrations of co-extractives are reduced. Thirdly, the compound must not undergo degradation during the gas-liquid chromatographic step. All these are obvious factors that require consideration if accurate results are to be obtained, and yet it is surprising how many examples of failures to check these points thoroughly are to be found in the literature. The effect of deficiencies in the method corresponding to these three factors is to give results with a negative bias, *i.e.* the reported concentrations are lower than the true values.

There is a second type of error which results in a positive bias. This is the presence in the sample of one or more compounds that behave in a similar manner to the compound of interest (A). The clean-up procedures used to reduce the relative concentrations of co-extractives do not have strong discriminatory power and thus the ability of the gas-liquid chromatographic

Scheme 1. Some organochlorine compounds used in industry, agriculture and medicine.



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procedure to separate A from all other compounds with a similar polarity is of great importance. This specific problem becomes more pressing, and is increasingly difficult to solve, as the concentration of A in the sample decreases. Before it can be discussed, the concept of chemical identity and methods of proof of identity must be considered.

In classical organic macroanalysis two concepts, together with their appropriate operational procedures, are used. These are class and identity. The latter is of primary importance and, as far as organic chemistry is concerned, may be defined as a unique chemical structure which has a set of unique and characteristic properties. Thus identification is defined as the assignment of a unique chemical structure to the molecules constituting an entity which is considered to be chemically pure and homogeneous. The deductive reasoning by which a particular structure is assigned to the molecules of the chemically pure entity consists of two experimental processes; degradation of the complex molecule by defined procedures to simpler molecules with established structures; and synthesis of the various structures compatible with the degradation studies and comparison of the properties of the various synthetic products with those of the unknown compound.

When we consider the type of analytical evidence used as a basis for deciding that component X in an environmental sample is a compound A of known structure, it is immediately apparent that the classical organic macroanalytical procedures of classification and identification are not used, and, as regards identification, are not even applicable. In the majority of investigations one or more of four types of parameters have been used to identify trace amounts of organochlorine compounds: (*i*) gas–liquid chromatographic retention times using two or more stationary phases, (*ii*) thin-layer chromatographic R_f values using one or two systems, (*iii*) preparation of derivatives and matching of the gas–liquid chromatographic retention times (and/or thin-layer chromatographic R_f values) of the products with those of known compounds, (*iv*) partition p-values. Other information, less commonly used, is replacement of the electron-capture detector by others such as the microcoulometric detector, and examination of samples which were collected before the introduction of a particular compound and which were stored in such a manner as to prevent subsequent contamination.

None of these procedures is sufficient of itself or in combination with the other procedures to establish the identity of a component in an environmental sample. Where, for example, samples pre-dating the introduction of a particular compound A are used, the presence in recent samples of a compound X with the gas–liquid retention time(s) similar to those of A, and the absence of such a component in the pre-samples is not proof that X is A unless it is shown that no other compound has recently come into use that has gas–liquid chromatographic characteristics similar to those of A. Strictly, the use of a combination of observations is an example of recognition and not of identification.[6] The structure of the argument is as follows.

The unknown component has m characteristics of which $p_1, p_2 \ldots p_i$ have been determined. A compound of known structure has n characteristics of which $P_1, P_2 \ldots P_i$ have been determined. It is found that $p_1 = P_1$, $p_2 = P_2$, $\ldots p_i = P_i$. The inference that 'X is A' is valid if, and only if, the one-to-one matching of the properties p_i and P_i, p_2 and P_2 *etc*, entails that the properties p_{i+1} and P_{i+1}; p_{i+2} and P_{i+2} *etc*., are also matched pairs and that $m = n$. But this necessary condition cannot be inferred from the evidence available. It may be concluded, given the set of i properties, that X is probably A, but the degree of confidence attached to this probability is intuitive and is not objectively established. Further, the probability that the conclusion is correct

Scheme 2. Metabolites of some organochlorine compounds.

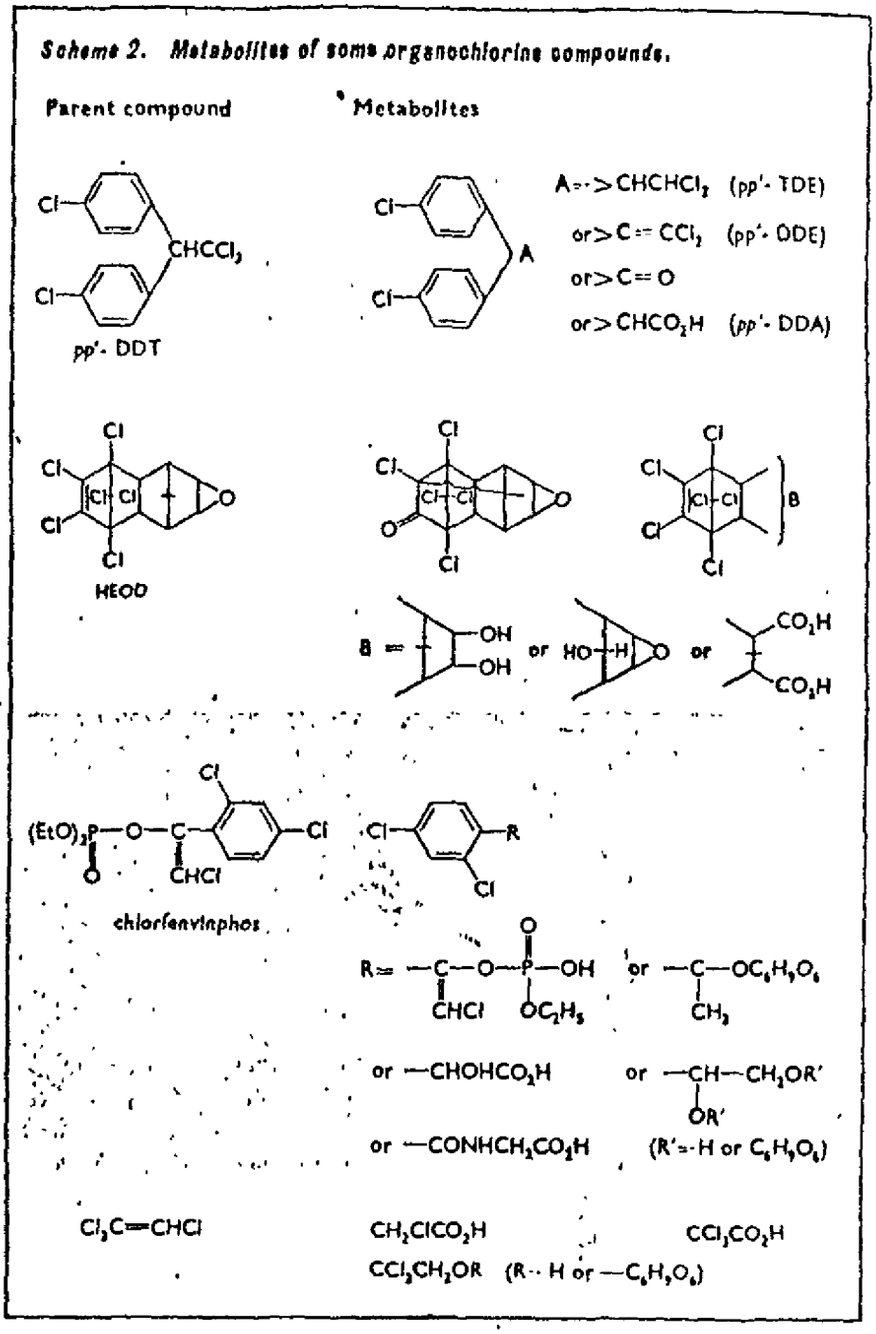

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Table 1. Mean concentrations (in ppm) of some organochlorine compounds in various environmental samples.

Type of sample	DDT and related compounds	Dieldrin
Riverwater (US)	8×10^{-6}	7×10^{-6}
Air (UK)	13×10^{-6}	21×10^{-6}
Rainwater (UK)	79×10^{-6}	8×10^{-6}
Sewage effluent (UK)	131×10^{-6}	145×10^{-6}
Human blood (UK)	1.5×10^{-3}	3×10^{-6}
Milk (UK)	3.5×10^{-3}	2×10^{-3}
Human fat (US)	10.30	0.22
Birds' eggs (UK)		
Rook	0.15	0.13
Kittiwake	0.53	0.09
Golden Eagle	0.49	0.34
Heron	5.50	2.20
Sparrowhawk	31.40	4.60

is dependent on the independence of the parameters $p_1, p_2 \ldots$ etc., and in this regard the significant correlations between gas-liquid chromatographic retention times on different stationary phases reduces the usefulness of these particular parameters.

The important topics of chemical identity and the recognition of micro- and submicro- quantities of organochlorine compounds have been outlined in some detail since many ecologists and conservationists do not seem to understand the analytical difficulties that occur during environmental surveys (and some toxicologists still use the concept of zero residues). Some of the results reported are summarized in Table 1.

Degradation

Another aspect of the organochlorine compounds, which is important in relation to their dispersal and tendency to concentrate in particular components of the environment, is their chemical and biochemical degradation. In recent years, it has become apparent that these compounds are more susceptible to degradation, particularly in animal systems, than had been realized earlier; in some cases the rates of conversion are slow, and this, together with slow rate of transfer to the sub-systems containing the appropriate enzymes, has resulted in their being regarded as 'persistent'. Once again, the need for precision in the definitions of concepts must be stressed: a persistent entity is one which undergoes no change, and to apply the adjective to an entity which undergoes change, even if only slowly, is incorrect.

It is more accurate to say that the organochlorine compounds undergo slow biochemical breakdown, the rates being such that the time required for a 50 per cent change is of the order 0.05–2 years. Rates of degradation in the abiotic components of the biosphere—soil, water, air—may be much slower, but there is no evidence that any of the organochlorine compounds used as insecticides, dielectric fluids etc., can be regarded as persistent in a manner analogous to the inorganic elements which are involved in the geochemical cycles.

Although the organochlorine compounds used in agriculture, industry etc., are susceptible to biological degradation, the structures of the majority of the metabolites indicate an underlying problem which, as yet, has received little attention—what is the fate of the metabolites themselves? *Scheme 2* shows that the known metabolites of organochlorine compounds, with the exception of a few simple chloroalkanes, often contain as many chloro-substituents as the parent compound. This phenomenon is probably a result of the relative stability of the C–Cl bond already referred to, and we may speculate that in the parent compounds the rate of conversion of C–H bonds to C–OH etc., is much faster than any reaction at the C–Cl bond. Further, once the polar metabolites are formed, the rate of reaction of a C–Cl bond in such metabolites is probably slower than in the parent compound. Thus the polar metabolites will probably approximate more closely than the parent compounds to the persistent character of the inorganic constituents of the biosphere and, as a result of their increased hydrophilicity, they may tend to accumulate in the hydrosphere. As the hydrosphere is an important source of food, and plays an important role in the control of the concentration of carbon dioxide in the atmosphere, this possibility requires careful study. Fortunately, the biological activities of these hydrophilic metabolites appear to be relatively low, and so there is sufficient time to carry out detailed studies to assess the corrective measures required and to balance these against other objectives.

Summary

Increasing attention is being given, very rightly, to the study of the pollution of the environment by chemicals. Investigations of the concentrations of organochlorine compounds in various parts of the biosphere have played a major role in stimulating concern about the effects on the environment not only of these compounds but also of the manufacture, use and disposal of all chemicals. The development of highly sensitive analytical techniques for organochlorine compounds has assisted in the study of their dispersal in the biosphere, but there are many analytical problems still awaiting solution, particularly with regard to the concept of chemical identity at the submicro level. Studies of the fate of organochlorine compounds, both as regards the routes and rates of degradation, are also giving insights which will be invaluable to the toxicologists and ecologists in their assessment of the current or future potential hazards of chemical pollution. This information, together with that from other scientific disciplines, will enable us to make rational decisions of the cost-benefit type and to forestall irreversible ecological damage by chemicals.

References

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Diagnosing lead poisoning

Lead poisoning can cause anaemia, brain damage or death—so a new fast accurate technique for measuring blood lead concentrations, devised by Dr H. T. Delves of Great Ormond St Hospital, could be a life saver. In recognition of his work, Perkin Elmer, which is now marketing the Delves Microsampling Cup, has given $1000 for research in his department. In traditional atomic absorption spectroscopy, only a small part of the sample ever gets into the light beam. In the new method, which uses only 10 µl of blood, the partially oxidized blood is placed in the flame in a nickel crucible and the atomized sample collects in a hollow cylinder and is thus concentrated in the light beam. At 30–50 analyses per hour, the method could be used for mass screening for lead poisoning.



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