This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of either the World Health Organization or the United Nations Environment Programme

Environmental Health Criteria 9

DDT AND ITS DERIVATIVES

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Division of Environmental Health, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

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ENVIRONMENTAL HEALTH CRITERIA FOR DDT AND ITS DERIVATIVES

A WHO Task Group on Environmental Health Criteria for DDT and its Derivatives met in Geneva from 8–14 November 1977. Dr V. B. Vouk, Chief of the Control of Environmental Pollution and Hazards Unit opened the meeting on behalf of the Director-General. The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks from exposure to DDT and its derivatives.

Dr W. J. Hayes, Jr and Dr J. Robinson, Sittingbourne Research Centre, Kent, England, assisted the Secretariat in preparing the first and second drafts of the DDT criteria document. Comments on which the second draft was based were received from the national focal points for the WHO Environmental Health Criteria Programme in Australia, Belgium, Canada, Finland, France, Greece, Israel, New Zealand, Pakistan, and USA and from the International Agency for Research on Cancer (IARC), the International Labour Office (ILO), the International Union of Biological Sciences (IUBS), the International Union of Pure and Applied Chemistry (IUPAC), the United Nations Industrial Development Organization (UNIDO), and from the United Nations Environmental Programme International Register of Potentially Toxic Chemicals.

Comments were also received from Dr V. Benes, Czechoslovakia, Dr S. Gabor, Romania, and Dr P. M. Newberne, USA.

Two subgroups reviewed the major part of the second draft (sections 2 to 6 and 7 to 8, respectively) and their comments were accepted as those of the whole group. Sections 1 and 9 were redrafted and approved at the plenary sessions.

The collaboration of these national institutions, international organizations, WHO collaborating centres, and individual experts is gratefully acknowledged. Without their assistance this document would not have been completed. The Secretariat wishes to thank, in particular, Dr W. J. Hayes, Jr for his help in all phases of preparation of the document.

This document is based primarily on original publications listed in the reference section. However, several comprehensive reviews on the health effects of DDT have also been used including publications by the US Environmental Protection Agency (1975), Müller (1959), and Mrak (1969).

Although the ecological aspects of DDT, including its possible accumulation in some components of the food chain, its metabolism in microorganisms and plants, as well as its effects on terrestrial and aquatic ecosystems are, no doubt, of great interest and importance, this document is concerned mainly with the discussion of its metabolism and effects in

experimental animals and man that have direct implications for human health.

Details of the WHO Environmental Health Criteria Programme including some of the terms frequently used in the documents may be found in the general introduction to the Environmental Health Criteria Programme published together with the environmental health criteria document on mercury (Environmental Health Criteria 1—Mercury, World Health Organization, 1976), now also available as a reprint.

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER STUDIES

1.1 Summary

1.1.1 Properties and analytical methods

DDT which is an acronym for dichlorodiphenyltrichloroethane is the prototype of broad action, persistent insecticides. It is stable under most environmental conditions and is resistant to complete breakdown by the enzymes present in soil microorganisms and higher organisms. Some of its metabolites, notably 1,1'-(2,2-dichlorethenylidene)-bis[4-chlorobenzene] (DDE), have a stability equal to, or greater than that of the parent compound. The persistence of DDT and DDE in the environment is mainly due to the fact that they are soluble in fat and virtually insoluble in water.

Two techniques have played a major role in the quantitative analysis of DDT-type compounds. The original Schechter-Haller colorimetric method introduced in 1945 was modified in 1953 making it possible to measure both DDT and DDE in the same sample. A second more reliable and versatile method for the simultaneous analysis of DDT, DDE and a number of other organochlorine insecticides began to be used extensively in about 1962. This consisted of gas–liquid chromatography with destructive and non-destructive detector systems using multicolumns for the separation of mixtures. Both methods require understanding and care in the selection, extraction, clean-up, and subsequent analysis of samples. Later, gas–liquid chromatography was combined with mass spectrometry, which added a dimension of mass for each component of a mixture and provided a more reliable technique for confirmatory analyses.

Analytical methods, their execution, and the reported results have not been satisfactory in a number of papers. However, good agreement has been achieved by analysing paired aliquots by the colorimetric and gas chromatographic method. In the majority of cases, analytical errors involving human samples have been small compared with the real differences, either between individual samples or between groups of samples drawn from populations with substantially different histories of exposure. This situation is different with environmental samples where misinterpretation can occur more often.

1.1.2 Production and uses

Synthesis of DDT was reported in 1874 but its effectiveness as an ecticide was not discovered until 1939. Because of limited supplies, most

of the compound produced in the world was devoted first to protection of military areas and personnel, mainly against malaria, typhus, and certain other vectorborne diseases. Even in 1944, only 4366 tonnes of DDT were produced in the United States of America. The following year, production reached 15 079 tonnes and, on 31 August 1945, DDT was released for commercial sale. Widespread agricultural use dates from 1946 in the USA and slightly later in most other countries.

In the USA, use increased until 1959 (35 771 tonnes) and then declined gradually so that only 13 724 tonnes were used in 1969. However, because of the export market, production continued to increase until 1963 (81 154 tonnes) and then this too gradually decreased. Unfortunately, there does not appear to be a continuous record of world production of DDT but according to figures supplied to the Organization for Economic Co-operation and Development (OECD), worldwide production in 1974 was 60 000 tonnes. It is known that DDT has been manufactured in many parts of the world including the developing countries. However, at present there is only one factory in the USA, one in France, and one in India.

The ban on the use of DDT and certain other organochlorine insecticides in Sweden from 1 January 1970 was based on a number of ecological considerations. More recently a number of other developed countries have restricted or banned the use of DDT except when it is needed for the protection of health. However, DDT is still used extensively for both agriculture and vector control in some tropical countries. If DDT were not used, vast populations would again be condemned to the ravages of endemic and epidemic malaria. Substitution of malathion or propoxur for DDT would increase the cost of malaria control by approximately 3.4- or 8.5-fold, respectively, and these increases could not be supported by some countries without decreasing the coverage of their control programmes.

1.1.3 Environmental concentrations and exposures

When sprayed, some DDT always fails to adhere to the target for which it is intended and drifts away. Vaporization from treated fields can be detected for more than six months after application. Most of it settles in the same area with an almost straight-line, inverse relationship to the logarithm of the distance from the source. However, some drift is worldwide. Traces of DDT have been recovered from dust known to have drifted over 1000 km and in water melted from Antarctic snow. With rare exceptions, the concentration of DDT in air in nonagricultural areas has been in the range of <1 to 2.36×10^{-6} ng/m^3. In agricultural communities, concentrations have ranged from 1 to 22×10^{-6} mg/m^3. In communities with anti-mosquito fogging programmes, concentrations of DDT may be much higher, 8.5×10^{-3} mg/m^3 being the highest level recorded.

Although very difficult to measure, concentrations of DDT in rainwater have usually been of the same order of magnitude (1.8×10^{-5} to 6.6×10^{-5} mg/litre) in both agricultural areas and very remote nonagricultural areas, suggesting that the compound is rather evenly distributed in the air. Presumably because of dust, a maximum concentration of 4×10^{-4} mg/litre was found in rainwater in an urban area. Concentrations of DDT in surface water depend on the soil as well as on rain. The concentrations in the USA are said to have reached a peak in 1966 and then dropped sharply. The highest level ever detected in potable water (2×10^{-2} mg/litre) was reported in 1960. In recent years, all concentrations have been $<1 \times 10^{-3}$ mg/litre and average concentrations have been similar to those for rainwater.

Because of drift, DDT concentrations of 0.10–0.90 mg/kg found in the soil of pastures and other fields not treated with insecticide were only a little less than those in the soil of cultivated fields that had been treated with DDT for 10 years or more (0.75 to 2.03 mg/kg). Most of the compound was in the upper 2.5 cm of soil. Due to evaporation, the total residue of DDT in soils treated for 10 or more years is of the same order of magnitude as that found soon after a single application at the same annual rate.

Daily intake of DDT from food has been measured in several countries. In the USA during 1953–54, average daily intakes of DDT and of total DDT[a] were 0.184 and 0.286 mg/man, respectively, most of which originated from foods of animal origin. Ten years later, following restrictions with regard to the application of DDT to livestock, their barns and forage, and to crops eaten directly by people, the same investigators found daily intakes of 0.038 and 0.087 mg/man for DDT and total DDT, respectively. The so-called Market Basket Survey showed a gradual decrease in daily intake of DDT to 0.015 mg/man in 1970. Intake in Canada and the United Kingdom was slightly less for comparable periods. In many countries of Europe and in other countries with similar diets, the intake of DDT has been judged to be about the same because of the similarity of diet and of measurements of the compound in staple foods and other important items of the diet. There is a need to measure total intake of DDT with food in some countries where this has not been estimated. Vegetarians generally consume less DDT than people who include meat in their diet, and local practices, including the practices of individual farming families, may greatly influence the DDT intake of the persons involved. Extensive use of DDT in the home may contribute moderately to intake but whether this increased intake is via food is unclear.

With few exceptions, the highest average concentration of DDT in the air to which workers are exposed (about 7 mg/m^3) is that associated with

[a] Total DDT is a term used to include both DDT and its metabolites DDE and TDE (DDD).

spraying the inside of houses as for malaria control. However, concentrations as high as 104 mg/m^3 have been reported in places where DDT was prepared and packed. Almost all of the DDT in the air of workplaces is in the form of aerosols. Because of particle size and other factors, the amount of DDT that workers may inhale is far less than the amount reaching unclothed portions of their skin. This is probably important even though DDT is less easily absorbed through the skin than many other organochlorine insecticides.

More knowledge concerning exposure of workers has been gained from measurements of the storage of DDT in the body and its excretion than from environmental measurements. Studies on volunteers have made it practical to determine intake from either storage or excretion values. In making these studies on workers, advantage has been taken of groups employed full time in the manufacture, formulation, or application of DDT and of others who were in contact with the material intermittently, sometimes only for a few hours per day and for a few weeks per year. In the literature, full-time exposure has been referred to as "heavy" but usually without any intention of implying that it was excessive or harmful. In fact, improved occupational safety and health measures have made it possible to reduce the rate of absorption associated with occupational exposure.

1.1.4 Metabolism

DDT is absorbed after inhalation and ingestion, the latter being the more important route of absorption. Absorption of large doses is facilitated by solution in animal or vegetable fat; absorption of small doses, such as those found in the residues of food, is virtually complete and is facilitated by the presence of fat in food. Even in solution, DDT is poorly absorbed through the skin.

Most of the known facts concerning the distribution, storage, and excretion of DDT have been demonstrated in man as well as in animals. The compound is stored preferentially in fat, and its storage in organs and other tissues following repeated intake is proportional to the neutral fat content of the tissues. However, uptake of DDT by fat is slow, thus much more is distributed to other tissues following a single, large dose and much more to adipose tissue following many small doses. In spite of the affinity of DDT for adipose tissues, most of the DDT-related compounds in blood are carried by proteins, less than 1% being carried in the tiny droplets of fat normally present in the blood.

Following repeated doses, storage in adipose tissue increases rapidly a first and then more gradually until a steady state is reached. In each speci

the height of the plateau is proportional to the dosage;[a] however, storage is relatively less at higher dosages because excretion is relatively greater. In man, the time necessary to reach storage equilibrium is at least one year. There is a gradual reduction in the amount of DDT stored in the tissues, if exposure to the compound is discontinued.

Like most species, man converts some DDT to DDE, which is stored even more avidly than the parent compound. A small amount of 1,1'-(2,2 dichloroethylidene) bis [4-chlorobenzene] (TDE, DDD) an intermediate in the formation of the main excretory product 2,2-bis(4-chlorophenyl)-acetic acid (DDA), may also be found in tissues. A number of other metabolites have been demonstrated in animals but not detected in man. Technical DDT is more readily excreted and less readily stored than *p,p'*-DDT because it contains 15–20% of *o,p'*-DDT.

DDT dosage-effect relationships have been measured in man by studying storage and excretion in the general population and in volunteers. Studies of total diets in the general population revealed intakes ranging from about 0.02 to 0.20 mg/man per day, in different subpopulations. In studies on volunteers, dosage was administered under supervision at the rates of 3.5 and 35 mg/man per day. The steady-state level of storage in the fat of the volunteers who received 3.5 mg/man per day was about 50 mg/kg while that of those receiving 35 mg/man per day was about 300 mg/kg. In recent years, the concentrations of DDT and of DDT-related compounds stored in adipose tissues in most populations have averaged <5, and <15 mg/kg, respectively. Higher values have been found where DDT was used extensively and without restriction in agriculture or was added directly to staple foods to control insects. In England and some other countries where cool weather and a short growing season help to control insects, the average concentrations of DDT and total DDT stored in adipose tissues have been <2 and <5 mg/kg, respectively. In any country, the nonoccupational exposure to DDT and, thus, the concentrations stored, may vary between subpopulations.

Most reports of the concentrations of total DDT in the blood of the general population of different countries lie within the range 0.01 to 0.07 mg/litre. The highest single value reported was 0.336 mg/litre and the highest average value was 0.136 mg/litre. The concentrations of DDT in the blood and other tissues of the fetus or newborn are lower than in corresponding tissues of the mother.

Concentrations of DDT in human milk have usually been reported to be in the range of 0.01 to 0.10 mg/litre with the concentration of DDT plus its

[a] The Task Group agreed that, for the purposes of this document, the term dosage should apply to any rate or ratio involving a dose, e.g., mg/kg, or (mg/kg)/day.

metabolites, especially DDE, about twice as high. However, in a few countries, average values for total DDT ranging from 1 to 5 mg/litre have been reported, the highest value observed being 12.21 mg/litre.

The average concentration of DDA in the urine of the general public is 0.014 mg/litre, only slightly less than the lowest concentration detectable by earlier analytical methods.

Occupational exposure commonly produces average concentrations of DDT and total DDT stored in fat ranging from 50–175 mg/kg and 100 to 300 mg/kg, respectively. The highest values recorded for DDT and total DDT in a healthy worker, whose exposure was not measured, were 648 and 1131 mg/kg, respectively. Typical concentrations of DDT and total DDT in the serum or plasma of workers with substantial exposures have ranged from 0.14 to 0.57 mg/litre and 0.35 to 1.36 mg/litre, respectively. The concentration of DDA in the urine of substantially exposed workers has been in the range of 0.5 to 3.0 mg/litre. Concentrations of DDT or its derivatives in fat, serum, or urine may be used to estimate the dose, if exposure has been prolonged and essentially steady. The ranges of storage and excretion, just mentioned, were measured in workers who were found to have absorbed a total dosage ranging from 0.25 to 0.5 (mg/kg)/day.

Animal studies indicate that the concentration in serum most accurately reflects the concentration in the brain, the critical tissue. In the rat, a level in the brain of 25 mg/kg is not usually fatal although higher levels tend to be.

1.1.5 Experimental studies of the effects of DDT

The toxicity of a single dose is affected by the solvent vehicle and representative median lethal dosage (LD_{50}) values for the rat are 250 mg/kg, for oral administration in oil, and 250–500 mg/kg or 3000 mg/kg for dermal administration in oil, or powder, respectively.

Large doses of DDT produce vomiting in man and other species that can vomit and this can modify the amount absorbed.

The main effect of DDT is on the nervous system. All parts, both central and peripheral, are affected to some degree. In animals, single or repeated doses can produce hyperexcitability, tremor, ataxia, and finally epileptiform convulsions. Ataxia may be demonstrated by functional tests in animals that have received daily dosages too small to produce noticeable clinical effects. Death is usually due to respiratory failure at the convulsive stage of poisoning. In some species, DDT sensitizes the heart to arrhythmia, which is made worse by epinephrine of endogenous or exogenous origin, and these animals die in ventricular fibrillation.

It appears that the mechanism of the toxic action of DDT is associated with its effect on the membranes in the nervous system. *In vit*

concentrations as low as 10^{-8} mol/litre change the movement of both sodium and potassium ions through the axonal membrane, and this movement is involved in the transmission of nervous impulses. Other evidence of nervous system effects are changes in the concentrations of 4-(2-amino-1-hydroxyethyl)-1,2-benzenediol (norepinephrine) and other neurotransmitters in poisoned animals.

Apart from the nervous system, the liver is the only other organ significantly affected by DDT. Potentially fatal doses of the compound cause focal necrosis of liver cells in several species. These lesions heal by autolysis and phagocytic action in animals that survive. A distinct form of liver cell change reflecting stimulation of microsomal enzymes is for all practical purposes confined to rodents. DDT induces microsomal enzymes in all species tested, but only in some rodents does the endoplasmic reticulum increase so much that the entire liver cell enlarges and granules that are normally scattered throughout the cytoplasm are displaced to the margin of the cell. These changes are accompanied by a moderate increase in fat droplets some of which become surrounded by whorls of endoplasmic reticulum to form so-called lipospheres. These characteristic changes have been observed by electron micrography as early as four days after administration of DDT, and may have occurred even earlier.

If DDT is fed for long periods at dietary levels ranging from 2 mg/kg upwards for mice or 5 mg/kg upwards for rats, the changes in the liver progress from hypertrophy, margination, and lipospheres in isolated, centrolobular hepatocytes to the formation of nodules of affected cells. The first change has been observed within 4 days of administration, the earliest time of observation. With administration of dosages corresponding to those that people may encounter, changes in the livers of susceptible rodents require the entire lifetime of the animal to develop fully. At first, the nodules are microscopic in size, but some may become more than a centimetre in diameter, particularly in mice, and show almost complete loss of lobular architecture. The same series of changes can be produced in rodents by other inducers of microsomal enzymes, including phenobarbital. Although there is persuasive evidence that these multinodular tumours of mice associated with changes in the endoplasmic reticulum are carcinomas, there is equally convincing evidence that they are not and the views of some highly qualified pathologists in this matter remain diametrically opposed. More important than the question of classification is the fact that the entire continuum of changes from the prompt response in isolated cells to the eventual formation of tumours is peculiar to some rodents, and does not occur in other animals in which the endoplasmic reticulum does not respond morphologically in the same way.

A number of enzymes of intermediate metabolism are either stimulated or

moderately inhibited by toxic doses of DDT; the possibility that these changes are the result rather than the cause of poisoning has not been excluded.

Levels of DDT as high as 200 mg/kg of food that do not produce any sign of poisoning, have not produced any adverse effects on fertility, gestation, viability, and lactation, and on the health of the progeny of rats and mice. Reproduction was normal in dogs receiving a dosage of 10 mg/kg body weight per day, which is approximately equivalent to a dietary level of 500 mg/kg for this species.

No teratogenic effects of DDT have been observed in multigeneration studies of reproduction in several animal species.

There is some uncertainty concerning the effects of DDT on the immune system; where an effect has been observed, it has been of a probiotic nature.

Except for the weak estrogenic properties of *o,p'*-DDT, the endocrine related effects of DDT and its analogues are confined to the adrenals and even these effects are now considered to be mainly secondary to microsomal enzyme induction in the liver.

DDT has not been found to be mutagenic in bacterial test systems, either without or with metabolic activation. The evidence from mammalian test systems, *in vitro* and *vivo* is inconclusive.

No specific antidote for DDT poisoning is known, but sedatives (especially phenobarbital) and ionic calcium are useful for treating poisoning in dogs and monkeys. Glucose or other ready sources of energy are also helpful in treatment.

1.1.6 Clinical and epidemiological studies on the effects of DDT

Mild poisoning was produced in one volunteer who ingested 750 mg DDT in oil in order to study its effects. All other poisoning of human subjects by DDT has been the result of accidental or suicidal ingestion. No systemic poisoning has resulted from occupational exposure to DDT, but a few workers have developed rashes or irritation of the eyes, nose, and throat associated with dust. Most of the very few fatal cases have involved children who drank solutions of the compound and whose clinical courses were dominated by solvent poisoning.

Signs of DDT poisoning in man are entirely similar to those observed in animals. In addition, persons poisoned have experienced a prickling sensation of the tongue and around the mouth and nose, reduction of tactile sense, paraesthesia of the extremities, nausea, dizziness, confusion, headache, malaise, and restlessness. In most patients, all signs and symptoms (including vomiting) probably involved the nervous system; a few had temporary jaundice indicating liver injury. In the majority of survivo

recovery was well advanced in 24 hours but a few required a week or more. Three men still had some weakness and ataxia in their hands five weeks after ingesting an amount estimated to be as high as 20 000 mg of DDT per person.

Stimulation of microsomal enzymes of the liver has resulted from full-time occupational exposure and from the therapeutic use of DDT in the treatment of familial, nonhaemolytic, unconjugated jaundice.

The only demonstrated effects of DDT on the general population are the storage of the compound and some of its derivatives in the tissues and their excretion in urine and milk. No confirmed ill-effects of DDT have been reported in babies, even in communities where the highest concentrations of the compound in human milk have been observed.

Careful investigation of the largest available groups of workers who have been exposed for as long as 25 years to significantly higher levels of DDT than the general population, has not revealed any evidence that DDT causes cancer in man. The total number of people in the world who have had many years of full-time occupational exposure to DDT is smaller than might be supposed. This makes the detection of any effect with a low incidence difficult. While it has been recognized that some human carcinogens have been detected only after comparatively long periods of exposure, it is also known that others (e.g., 2-naphthylamine) have been detected through their occurrence in high incidence in small groups following exposure for periods of much less than 25 years.

1.1.7 Dosage-effect relationships

Dosage-effect relationships for DDT in man have been observed in connection with acute poisoning, excretion, and storage, and the induction of microsomal enzymes has been observed at a dosage of 0.25 (mg/kg)/day but not at lower dosages. The dosage of 0.25 (mg/kg)/day to which workers have been exposed for 25 years is of the same order of magnitude as the dosage that causes an increase in the incidence of tumours in male mice of a susceptible strain but not in females of any strain. (See section 1.2.4). This same dosage is lower than the nonobserved effect levels for rats, dogs, and monkeys and far less than the dosage at which rats, mice, and dogs continue to reproduce successfully for generations.

1.1.8 Evaluation of risk

Food represents the major source of intake of DDT for the general population. The average intake of DDT from all sources is unlikely to exceed 0.05 (mg/man)/day. Occupational exposure to DDT is mainly

respiratory and dermal. However, much of what is inhaled is deposited in the upper respiratory tract and subsequently ingested. The effects of dermal exposure are minimal because the compound is poorly absorbed through the skin; the excellent safety record, never matched by any other insecticide used in antimalaria campaigns, other vector control programmes, and agriculture is due mainly to this fact. The number of people throughout the world currently engaged in the manufacture of DDT is small and, wherever safety and health protection measures are good, occupational exposure of this group is minimal. Formulators and applicators are also groups that are occupationally exposed to DDT but there is no evidence to suggest that their intake is significantly higher than that of workers engaged in the manufacture of DDT.

No adverse effects have been described in man at repeated dosage of 1.5 (mg/kg)/day. The large number of measurements that have been made on samples from human populations do not throw any real light on the question of maximum-tolerated doses or concentrations, apart from highlighting the fact that the levels found in volunteers and workers which were higher than those in the general population were not associated with any adverse effects.

In the light of currently available information, there is no evidence that DDT is carcinogenic in man. Liver tumours are produced in mice and possibly in rats by DDT, DDE, and TDE but there is disagreement on the significance of these tumours. Studies in *in vitro* bacterial test systems have not shown any evidence that either DDT or DDE is mutagenic. The evidence from mammalian test systems, both *in vitro* and *in vivo*, is inconclusive.

DDT induces microsomal mixed function oxidases in many animal species and causes marked morphological changes in the liver of some rodents. At the present time, it is very difficult to assess the biological significance of this effect for men, since the intake by members of the general population is much lower than the smallest daily dosage required to produce such an effect in man and animals.

In both man and animals, there is no indication that DDT affects reproduction or produces teratogenic effects although it has been shown to be embryotoxic in high doses.

DDT appears to have a depressant effect on the immune system although the evidence is by no means conclusive.

Animal studies indicate that nutritional status influences the toxicity of DDT. In man, nutritional status will have a similar effect to that in animals. However, the possibility that starvation in man could precipitate toxic manifestations is regarded as unlikely as the stored levels do not approach those found in laboratory animals.

The information derived from human exposure is insufficient to construct a comprehensive picture of the dosage-effect relationships for man except in connection with storage and excretion of the compound and its metabolites.

1.2 Recommendations for Further Studies

DDT is the first synthetic pesticide to which many people have been exposed to a measurable degree for a period of many years. It has already been the subject of an enormous amount of scientific study, but of course there is still more to be learned. The following recommendations are considered to have special implications for human health. Much other important biomedical research such as the continuing use of DDT as a tool for studying the nervous system has been excluded from this document.

1.2.1 Fate in the environment

There is a serious gap in knowledge of the circulation and fate of DDT and its analogues in the environment as a whole. Because this is directly connected with the assessment of future exposure pathways for man, the behaviour and fate of DDT in the environment should be studied more extensively. Great progress has been made recently in demonstrating the breakdown of DDT to carbon dioxide and hydrochloric acid under laboratory conditions similar to those found in the upper atmosphere. There is a need for further study of this phenomenon in the laboratory, especially using DDT labelled with ^{14}C and ^{3}H. There is an even greater need for seeking quantitative information on the rate at which photomineralization may occur in nature and the factors that influence this rate.

1.2.2 Monitoring of exposure and effects

There are fairly accurate estimates of the daily intake of DDT in several developed countries. In other countries where DDT is most likely to be used continuously, the daily exposure of the general population to DDT in food should be monitored, especially if there are indications that the conditional acceptable daily intake (ADI) of 0.005 (mg/kg)/day might be exceeded. For comparison, monitoring programmes should be continued in countries where figures are available for earlier years.

Extensive information is available on the occurrence of DDT and its metabolites in human fat, blood, and milk. Continued, but limited monitoring is justified in order to learn the rate at which concentrations decline following a progressive reduction in the use of the compound. More

extensive monitoring is justified in countries where base data are not available and where the use of the insecticide is essential. Particular attention should be given to people with substantial occupational exposure. If values are found that are not consistent with the dietary and occupational history of each group, the cause of the variation should be sought. If values are unexpectedly high, some improper use may be discovered. If values are unexpectedly low, some modifying factor such as previously unrecognized intake of phenobarbital may be revealed.

Clinical studies should be made on any person or group found to have exceptional levels of storage or excretion in the hope of learning whether the insecticide has had any influence on their health or, conversely, to learn whether their nutritional state or the presence of chronic disease has interacted in any way with storage and excretion. Obviously such studies must be adequately controlled if the results are to be of use.

1.2.3 Carcinogenicity

Attention should be turned from the narrow question of the tumorigenicity of DDT in the liver of mice and rats to the broader question of the basis for this action of DDT and phenobarbital. A far wider range of inducers should be studied, keeping in mind that some inducers may have other important properties related to the initiation of tumours. Compounds belonging to several classes (e.g., an organochlorine insecticide, phenobarbital, a pyrethrin, etc.) should be studied in several species (including one nonrodent) to determine the dosage-response relationships for: (*a*) microsomal enzyme activity; (*b*) typical morphological changes in the endoplasmic reticulum of hepatocytes; and (*c*) liver tumours. These studies together with continuing epidemiological investigations of the effects of the same classes of compounds on people should make it easier to extrapolate from tumorigenesis in animals to the problem of cancer in man.

1.2.4 Mutagenicity

There is satisfactory evidence that DDT is not mutagenic in bacterial systems, without and with metabolic activation. The evidence derived from mammalian test systems, both *in vitro* and *in vivo* is inconclusive and should be clarified. Methods of mutagenicity testing are advancing rapidly, and shorter and possibly more sensitive mammalian tests are becoming available. A fresh evaluation of the mutagenicity of DDT in animals would facilitate further assessment of its significance for man.

2. PROPERTIES AND ANALYTICAL METHODS

2.1 Physical and Chemical Properties of DDT and Certain Related Compounds

2.1.1 Properties of DDT

The term DDT is generally understood throughout the world and refers to 1,1′-(2,2,2-trichloroethylidene)-bis(4-chlorobenzene) (*p,p′*-DDT). The structure of DDT permits several different isomeric forms, an example of which is 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*o,p′*-DDT). The term DDT is also applied to commercial products consisting predominantly of *p,p′*-DDT together with some *o,p′*-DDT and smaller amounts of other compounds. A typical example of technical DDT had the following composition: *p,p′*-DDT, 77.1%; *o,p′*-DDT, 14.9%; *p,p′*-TDE, 0.3%; *o,p′*-TDE, 0.1%; *p,p′*-DDE, 4.0%; *o,p′*-DDE, 0.1%; and unidentified compounds, 3.5%.

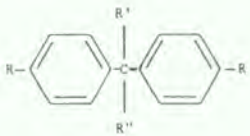
All isomers of the compound DDT are white, crystalline, tasteless, almost odourless solids with the empirical formula $C_{14}H_9Cl_5$ and a relative molecular mass of 354.5. The melting range of *p,p′*-DDT is 108.5–109.0° C and its vapour pressure is 2.53×10^{-5} Pa (1.9×10^{-7} mm Hg) at 20° C. DDT is soluble in organic solvents as follows (g/100 ml): benzene, 106; cyclohexanone, 100; chloroform, 96; petroleum solvents, 4–10; ethanol, 1.5. It is highly insoluble in water.

p,p′-DDT is dehydrochlorinated to form DDE (see Table 1) at temperatures above the melting point, especially in the presence of catalysts or light. Solutions in organic solvents are dehydrochlorinated by alkali or organic bases. Otherwise, DDT formulations are highly stable. The compound is also relatively resistant to breakdown by the enzymes found in soil and higher organisms, and DDE is even more resistant. Under simulated atmospheric conditions, both DDT and DDE decompose to form carbon dioxide and hydrochloric acid.

2.1.2 Properties of DDT analogues

The chemical structure of some of the analogues of DDT is shown in Table 1. The structure of the *o,p′*- and *m,p′*-compounds can be inferred from those of the *p,p′*-isomers. The table is confined to compounds that occur in commercial DDT, metabolites formed from them, and analogues that have had some use as insecticides. It must be emphasized that even the commercially-available insecticidal analogues have strikingly different

Table 1. Structure of *p,p'*-DDT and its analogues of the form:



(many of the compounds also exist as *o,p'*-isomers and other isomers)

Name DDT and its major metabolites	Chemical name	R	R'	R''
DDT	1,1'-(2,2,2-trichloroethylidene)-bis[4-chlorobenzene]	–Cl	–H	$-CCl_3$
DDE[a]	1,1'-(2,2-dichloroethenylidene)-bis[4-chlorobenzene]	–Cl	None	$=CCl_2$
TDE(DDD)[a,b]	1,1'-(2,2-dichloroethylidene)-bis[4-chlorobenzene]	–Cl	–H	$-CHCl_2$
DDMU[a]	1,1'-(2-chloroethenyldene)-bis[4-chlorobenzene]-	–Cl	None	$=CHCl$
DDMS[a]	1,1'-(2-chloroethylidene)-bis[4-chlorobenzene]	–Cl	–H	$-CH_2Cl$
DDNU[a]	1,1'-bis(4-chlorophenyl)ethylene	–Cl	None	$=CH_2$
DDOH[a]	2,2-bis(4-chlorophenyl)ethanol	–Cl	–H	$-CH_2OH$
DDA[a]	2,2-bis(4-chlorophenyl)-acetic acid	–Cl	–H	$-C(O)OH$
Some related insecticides				
Bulan®	2-nitro-1,1-bis-(4-chlorophenyl)butane	–Cl	–H	$-CH(NO_2)C_2H_5$
Prolan®	2-nitro-1,1-bis-(4-chlorophenylpropane	–Cl	–H	$-CH(NO_2)CH_2$
DMC	4-chloro-α-(4-chlorophenyl)-α-(methyl)benzenemethanol	–Cl	–OH	$-CH_3$
dicocol (Kelthane®)	4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzenemethanol	–Cl	–OH	$-CCl_3$
chlorobenzilate[c]	ethyl 4-chloro-α-(4-chlorophenyl)-α-hydroxybenzeneacetate	–Cl	–OH	$-C(O)OC_2H_5$
chloropropopylate[c]	1-methylethyl 4-chloro-α-(4-chlorophenyl)-α-hydroxy-benzeneacetate	–Cl	–OH	$-C(O)OCH(CH_3)_2$
methoxychlor[c]	1,1'-(2,2,2-trichloroethylidene)-bis[4-methoxybenzene]	$-OCH_3$	–H	$-CCl_3$
Perthane®	1,1'-(2,2-dichloroethylidene)-bis[4-ethylbenzene]	$-C_2H_5$	–H	$-CHCl_2$
DFDT	1,1'-(2,2,2-trichloroethylidene)-bis[4-fluorobenzene]	–F	–H	$-CCl_3$

[a] Recognized metabolite of DDT in the rat.

[b] As an insecticide, this compound has the ISO approved name of TDE, and it has been sold under the name Rothane®; in metabolic studies the same compound has been referred to as DDD; as a drug, it is called mitotane.

[c] Common name approved by the International Organization for Standardization (ISO).

properties. Especially remarkable is the slow metabolism and marked storage of DDT and its metabolite DDE and the rapid metabolism and negligible storage of methoxychlor.

No attempt has been made to include in Table 1 the wide range of compounds that have been synthesized and studied in connexion with structure-activity relationships, often with the hope of emphasizing the good properties of DDT and reducing its undesirable properties. For a more extensive consideration of analogues, see Metcalf (1955).

The formation of metabolites is considered in section 6.4.

2.1.3 Formulations of commercial or technical DDT

Technical DDT has been formulated in almost every conceivable form including solutions in xylene or petroleum distillates, emulsifiable concentrates, water-wettable powders, granules, aerosols, smoke candles, charges for vaporizers, and lotions. Aerosols and other household formulations are often combined with synergized pyrethrins.

When used as a drug, DDT is called clofenotane (INN) or Dicophane (British Pharmacopoeia), Klorfenoton (Swedish Pharmacopoeia), Chlorophenothane (United States Pharmacopoeia). For research or reference it has been designated OMS 0016 and Ent. 1,506. DDT has been sold under a variety of tradenames, including: Anofex®, Cezarex®, Dinocide®, Gesarol®, Guesapon®, Guesarol®, Gyron®, Ixodex®, Neocid®, Neocidol®, and Zerdane®.

2.2 Analytical Procedures

Analytical procedures for determining residues of DDT-type compounds in environmental samples involve several steps including collection and extraction of the DDT-type compounds; removal of coextractives by appropriate clean-up methods; and quantification of *p,p'*-DDT and its analogues by a suitable technique. Each of these major steps is discussed later. It is appropriate, however, first to outline briefly the statistical criteria used to assess analytical methods, the estimation of the lower limit of detection of a method, and the procedure for confirming the chemical identities of the components measured.

2.2.1 Statistical criteria for assessing analytical methods

The overall reliability of an analytical method can be assessed using two criteria, namely, reproducibility and systematic error (or bias).

Reproducibility is both conceptually and practically the simpler criterion; it may be defined as "the quantitative expression of the random error associated with operators working in different laboratories, each obtaining single results on identical test material when applying the same method" (Institute of Petroleum, 1968). It may be quantitatively specified in various ways, and it is important to pay attention to the statistic (range standards, deviation, etc.) used in a particular study to represent the random error of an analytical method. If the results are normally distributed then the most efficient statistic measuring reproducibility is the standard deviation of a set of results (Nalimov, 1963; Youden & Steiner, 1975; Davies & Goldsmith, 1976). Care should always be taken to ensure that a particular statistic or statistical technique is appropriate for a given set of results, by, for example, testing for outliers or, if there are sufficient results, examining the distribution of the results, before characterizing the reproducibility of an analytical method.

There are two subdivisions of the reproducibility criterion, namely, replication i.e., two or more results, obtained by the same operator in a given laboratory using the same apparatus for successive determinations on identical test material, within a short period of time on the same day; and repeatability i.e., a quantitative expression of the random error associated in the long run with a single operator in a given laboratory obtaining successive results with the same apparatus under constant operating conditions on identical test material (Institute of Petroleum, 1968). Reproducibility is, in turn, a subdivision of the random error in the analysis of identical test material in different laboratories using different techniques or variations of a particular method. Examples of the assessment of the random errors found in the determination of *p,p'*-DDT and related compounds are given below.

The systematic error of a method is the deviation of the experimental results from the "true" values; such systematic error causes the differences between the nominal value and the experimentally-determined values to have predominantly the same sign (as opposed to the random errors, where the results are equally likely to be greater than or less than the true mean). Nominal or "true" values are available only in the case of fortified (spiked) samples, but whether such fortified samples are representative of actual (environmentally incurred) contamination is open to doubt in the case of some types of material. A rapid nonparametric test of systematic error can be made using the sign-test or the Wilcoxan signed ranks test (Conover, 1971).

It is common practice to calculate the "recovery" factor for an analytical method, i.e., the ratio of the mean observed value to the nominal value (usually expressed as a percentage), but the statistical significance of th

"recovery" factor should always be assessed. The ratio of the mean deviation to the standard error of the mean deviation is an appropriate method of testing the null-hypothesis that the difference between the nominal value and the observed mean is not statistically significant.

The term "total error" has been proposed for a function incorporating both the systematic error and the reproducibility (McFarren et al., 1970), and these authors also suggested three classes of total error corresponding to excellent methods, acceptable methods, and methods that are judged unacceptable. It is pertinent that McFarren et al. concluded that the total errors in one study of the determination of DDT-type compounds in water were unacceptable (>50% for *p,p'*-DDT, and *p,p'*-DDE, 24.0–53.6% for *o,p'*-DDT).

2.2.2 Limit of analytical detection

All analytical determinations have a lower limit corresponding to that quantity (Δg) of *p,p'*-DDT (or a related compound) which produces a response (Δr) that cannot be distinguished from response ($\Delta°r$) produced when no *p,p'*-DDT is present. The response $\Delta°r$ is generated by the materials, reagents, and instruments, used in the procedure, for example, the small voltage generated by electronic equipment, or the small absorption in a spectrophotometric method. These responses are known as "blank" or "noise", and their size depends on the presence of interfering components in the test material, the purity of the reagents, the cleanliness of the apparatus, and the design of electronic equipment (amplifiers, etc.). The concept of limit of detection is a statistical one and is related to the random variation of the response generated by a blank or control (Sutherland, 1965; Skogerboe & Grant, 1970; Kaiser, 1973). Currie (1968) has defined three limiting levels for use in analytical chemistry: "the net signal level" above which an observed signal may be reliably recognized; "the true net signal level" which may, *a priori*, be expected to lead to detection; and "the quantifiable level" at which the measurement precision is sufficient for the quantity present to be estimated satisfactorily. In many types of sample, the limit of detection is of rather academic interest as the concentrations of DDT-type compounds in samples are an order of magnitude greater than the limit of detection of a sensitive detector such as the electron-capture detector. However, in analyses of air and drinking-water, for example, it may be necessary to ascertain the limit of detection by a suitable statistical procedure.

Lower limits of detectability ($ng \cdot g^{-1}$) suggested in the US EPA Manual of Analytical Methods (Thompson, 1974) for DDT-type compounds using

gas–liquid chromatography are:

o,p'-DDE	Adipose tissue, 10; serum, 1
p,p'-DDE	Adipose tissue, 20; serum, 1
o,p'-DDT, *p,p'*-TDE, *p,p'*-DDT	Adipose tissue, 20; serum, 2

2.2.3 Confirmation of the identity of trace residues of DDT-type compounds

Confirmation of the identity of *p,p'*-DDT and related compounds in many types of environmental samples is not easy when the apparent amounts present are in the microgram and submicrogram range, since the classical procedures for the identification of organic compounds require the use of milligrams of the purified compound. A definition of chemical identity appropriate to trace analysis has been proposed (Robinson et al., 1966), a definition that may need revision in relation to the combined use of gas–liquid chromatography and mass-spectrometry. Chemical derivatization techniques for DDT-type compounds have been reviewed by Cochrane & Chau (1971). Other techniques, that are used routinely in the confirmation of the identity of DDT-type compounds include: determination of gas–liquid chromatographic retention times using polar and nonpolar stationary phases; thin-layer chromatography; paper chromatography; p-values; infrared microtechniques; carbon skeleton chromatography; conversion into dichlorobenzophenones; nuclear magnetic resonance; and X-ray diffraction. Tables of the relative retention times (aldrin = 1.00) of DDT-type compounds using nine liquid phases (Thompson et al., 1975) and three mixed liquid phases (Suzuki et al., 1975); gas–liquid chromatographic retention times for DDT-type compounds are also summarized by Yermakov (1972). Relative thin-layer chromatographic R_f values (*p,p'*-DDE = 1.00) on alumina using three solvent systems were reported by Thomas et al. (1968). Extraction p-values for DDT-type compounds using seven solvent systems are given in the US EPA Manual (Thompson 1974). Identification of DDT and its metabolites by a microinfrared technique has been studied by Sierwiski & Helrich (1967) and reference infrared spectra have been published (Chen et al., 1972). Asai et al. (1971) used carbon skeleton chromatography to differentiate polychlorinated biphenyls from DDT-type compounds. The conversion of DDT-type compounds into the corresponding benzophenones may be used to identify them in the presence of polychlorinated biphenyls (Miles, 1972), but this technique has its drawbacks as *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE all give the same *p*,

dichlorobenzophenone. The use of high resolution nuclear magnetic resonance spectroscopy (with a time averaging computer to increase sensitivity), for the confirmation of the presence of *p,p'*-DDT and *p,p'*-DDE in human adipose tissue (total concentration about 13 mg/kg) was studied by Biros (1970).

The combination of gas–liquid chromatography with mass-spectrometry is a powerful tool for the confirmation of identity of trace residues of DDT-type compounds. For example, Gordon & Frigerio (1972) used mass fragmentography and claimed identification of 10 pg *p,p'*-DDT; Schaeffer (1974) identified DDT-type compounds in fish using a fast scan mass-spectrometer to give a multiple mass spectrum for each of the overlapping peaks.

2.2.4 Sampling and extraction

Before discussing different kinds of samples, it must be noted that valuable information on many analytical problems may be found in the US EPA Manual (Thompson, 1974) and the FDA Manual (McMahon & Sawyer, 1977).

DDT-type compounds may be present in the air in vapour form or adsorbed on particulate matter. Glass-fibre filters are suitable for trapping the particulate matter (Stanley et al., 1971; Beyermann & Eckrich, 1974) and DDT in the vapour form may be trapped using impingers of the Greenburg-Smith type and a suitable nonvolatile solvent using ethylene glycol. Miles et al. (1970) reported a trapping efficiency of more than 99% for DDT. A three-trap system in series comprising a column containing glass cloth, followed by an impinger with hexylene glycol, and finally an alumina column was used by Stanley et al. (1971). They estimated that the collection efficiency (based on material balance studies) of this system for a mixture of aerosol and vapour was about 60% for *p,p'*-DDE, 95% for *p,p'*-TDE, and 100% for *p,p'*-DDT. Beyermann & Eckrich (1974) used glass wool for the trapping of aerosols, and a stainless-steel net coated with polyethylene glycol for trapping vapours. Trapping of organochlorine insecticides in the vapour phase using support-bonded silicones on various types of Chromosorb was considered to be quantitative by Aue & Teli (1971): DDT-type compounds were not examined but the trapping of lindane, aldrin, and heptachlor was considered satisfactory. A cross-linked polystyrene resin, Chromosorb 102, was used by Thomas & Serber (1974). These workers reported a collection efficiency of 98% for *o,p'*-DDT at a concentration of 15 ng/m^3. Herzel & Lahmann (1973) used silica gel as the support for various liquid phases and concluded that polyethylene glycol was the best absorbent for DDT-type compounds in the air. Absolute

calibration of the collection efficiency of aerosols and atmospheres for DDT-related compounds is difficult, but the studies that have been made indicate that impingers with ethylene glycol or hexylene glycol are probably the most efficient. The simplest empirical test of the efficiency of the trapping system is to use two (or more) impingers in series and analyse the liquid absorbents from the impingers separately. The ratio of the amounts present in the first and second impingers should be higher than 10:1. Detailed instructions for the sampling and analysis of air for pesticides (including DDT-type compounds) by a method that has been subjected to interlaboratory study are given in the US EPA Manual (Thompson, 1974).

For the determination of DDT-type compounds in water an uncontaminated container may be filled, but if the concentrations of DDT are likely to be extremely low (as would be expected in most potable waters), then drawing the water through a suitable trapping device may be more appropriate; this method also gives a time-weighted average concentration and can be used in an automated monitoring procedure.

Benzene was used by Pionke et al. (1968) as the solvent to extract *p,p'*-DDT and *p,p'*-TDE from fortified samples of distilled water and lake waters. The levels of fortification were high (μg/litre) and the recoveries were: *p,p'*-DDT, 96.1% ± 1.02%; *p,p'*-TDE 97.3% ± 0.89%. Thus, at concentrations of the order of 0.001 mg/litre, the benzene extraction method gives excellent recoveries (total errors, 5.9% and 4.5%, respectively). A continuous flow method based on liquid–liquid partition was developed by Ahling & Jensen (1970); water is passed through a column containing Chromosorb W coated with undecane plus a macrogol Carbowax 4000 monostearate. The best collection efficiencies were found when the two liquid absorbents were used at concentrations of 10% and 30%, respectively, on the solid support. At concentrations in the ng/litre range, the optimum recovery of known amounts of DDT-type compounds added to water were obtained with 1.5 g coated Chromosorb W per litre of water. Ahnhoff & Josefson (1974) used continuous flow liquid–liquid partition between water and cyclohexane (in three extractors in series). The extraction efficiencies from water containing 5 ng *p,p'*-TDE or 7.6 ng *p,p'*-DDT per litre were greater at a flow rate of 2 litre/h than at 5 litre/h being 90% and 98% for *p,p'*-DDT, and *p,p'*-TDE, respectively, with 92% and 93%, respectively, of the recovered compounds being found in the first extractor. A method using a solid adsorbent, a macroreticular resin, XAD-4, has been studied by Musty & Nickless (1974); at a flow rate of 8 ml/min through a column containing 2 g XAD-4, satisfactory recoveries of *p,p'*-DDT and three related compounds were obtained at concentrations of 2–10 ng-litre. Carbon is a very efficient adsorber of *p,p'*-DDT and *p,p'*-TDE from water (Rosen & Middleton 1959), but desorption from charcoal is difficult; furthermore, the recover

are not very satisfactory and this is attributed to chemical changes catalysed by carbon in contact with water rather than inefficient desorption (Eichelberger & Lichtenberg, 1971). Another solid adsorbent is polyethylene (Beyermann & Eckrich, 1973), but in the case of river water variable results were obtained because of the effects of other solutes. Taylor & Bogacka (1968) used petroleum ether to extract DDT from water; following a clean-up with acetonitrile partition and Florisil the overall recovery (using thin-layer chromatography) was incomplete (about 66%). The total error of this procedure is unacceptable. Benzene was used as the extraction solvent by Djatlovitskaja et al. (1972), and a general review of the determination of organochlorine insecticides (and other insecticides) in water has been published by Novikova (1973).

The need for scrupulous cleanliness in glassware and purity of reagents cannot be overstressed in the case of the determination of DDT-type compounds in air and water as the residues are usually so small; the use of electronic equipment with a low noise characteristic and constant checking of the response of detectors are also necessary.

A major difficulty in the determination of organochlorine insecticides in soils is the initial extraction of the residues because of a combination of factors, including the wide variation in soil types. Chiba (1969) emphasized the lack of precision in defining soil types. In his review article, he concluded that the most effective solvent systems for extraction of these compounds were mixtures of n-hexane/acetone (1 : 1 v/v) or chloroform/methanol (1 : 1 v/v), and that the moisture content of the soil should be at least 5%. The results of a study of the determination of organochlorine insecticides in three types of soil have been summarized by Woolson & Kearney (1969). Twelve laboratories participated using various extraction procedures. In one type of soil (a silty clay loam) that was fortified with *p,p'*-DDT at a concentration of 5 mg/kg, the amounts found in the different laboratories varied between 1.60 and 5.48 mg/kg. The results of 3 of the laboratories were discarded by Woolson & Kearney (1969), and the mean recovery of the other 9 laboratories was 79.3%, with a standard deviation of 42.2%. Wetting of the soil before extraction was considered to improve the recoveries, Soxhlet extraction appeared to be preferable to shaking with the solvent, and hexane/acetone (1 : 1 v/v) or hexane/isopropanol (3 : 1 v/v) appeared better extractants than other solvents.

A further collaborative study of the determination of organochlorine insecticides in 3 types of soil was reviewed by Woolson (1974). Although 12 laboratories participated, only 7 completed the study. All the laboratories used the same extraction, clean-up and quantification procedures, including premoistening of the soil with ammonium chloride solution at 0.2 mol/litre. The recoveries of *p,p'*-DDE, *p,p'*-TDE, *o,p'*-DDT and *p,p'*-DDT were

higher than 80%, with standard errors of 8–18%. These results are much more consistent than those of the previous study, and Woolson recommended that premoistening of the soil with ammonium chloride solution (0.2 mol/litre) followed by extraction with hexane/acetone (1 : 1 v/v) should be adopted for the determination of chlorinated hydrocarbon insecticides in soil.

Several solvents have been used for the extraction of DDT-type compounds from nonfatty foods such as vegetables, fruit, and cereals; acetonitrile, alone or mixed with water is used in the method of the Association of Official Analytical Chemists (AOAC) depending on the water content of the sample (Horwitz, 1975); Cieleszky et al. (1970) recommend Soxhlet extraction with diethyl ether.

Maceration of vegetables with a mixture of acetone and hexane was used by Sissons et al., 1968; the same mixed solvent was used for cereals, and root vegetables by Abbott et al., 1969 who used propan-2-ol in the case of fruit and green vegetables. Whiting et al. (1968), and Skrentny & Dorough (1971) considered a mixture of methanol and chloroform to be the most efficient extraction solvent for use with a macerator or a Soxhlet extractor. The acetonitrile extraction technique was examined by Zerber et al. (1971) for the extraction of DDT-type compounds from cereal products and feeding stuffs. Diethyl ether was used by Kučinski (1972) for extraction from canned vegetable products.

Extraction methods for fat-containing foods are given in the AOAC Method (Horwitz, 1975), the solvent used, methanol or petroleum ether, being dependent on the type of sample. Soxhlet extraction using petroleum ether is recommended by the Federal Health Office of the Federal Republic of Germany (Anon., 1974); Cieleszky et al. (1970) also recommended petroleum ether as a suitable solvent.

Smart et al. (1974) compared acetonitrile and dimethyl formamide as extraction solvents for apples, carrots, potatoes, and vegetables; a third solvent dimethylsulfoxide was compared with these two solvents for butter, cheese, and eggs.

Three body tissues or fluids have been analysed in many surveys, namely adipose tissue, blood (or serum), and mother's milk. The US EPA Manual (Thompson, 1974) recommends grinding a sample of adipose tissue with anhydrous sodium sulfate before extraction with petroleum ether; carbon tetrachloride has been used as a solvent (Mattson et al., 1953), but it is not appropriate if the final quantification step involves gas–liquid chromatography and a halogen sensitive detector. Extraction of DDT-type compounds from blood or serum using hexane has been described (Dale et al., 1966b), but this extraction procedure is inefficient, probably as a result of binding by serum proteins. Dale et al. (1970) investigated a procedure in

which the serum was treated with 97% formic acid before extraction with hexane; experiments using ^{14}C-DDT indicated that extraction with hexane alone gave results some 40% lower (based on ^{14}C activity) than when the serum was first treated with formic acid. A modified procedure in which whole blood was treated with 60% sulfuric acid, and then extracted with a hexane/acetone (9 : 1) mixture has been reported by Stretz & Starr (1973). Samples of blood spiked at 4 different levels with *p,p'*-DDT were analysed by 11 different laboratories; considerable discrepancies were found between laboratories and a further study of the method was considered desirable. Griffith & Blanke (1974) also investigated the sulfuric acid method, but a microcoulometric detector was used instead of an electron-capture detector. According to these workers, consistent recoveries of *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE were obtained in their laboratory but reproducibility between laboratories was not studied.

The US EPA Manual (Thompson, 1974) method for the extraction of DDT-type compounds from human milk involves an acetonitrile/hexane type extraction. A method for extraction from cow's milk, that makes use of the stability of *p,p'*-DDT and its derivatives in the presence of concentrated sulfuric acid, has been published by Coha & Nedic (1970); the mixture of milk and concentrated sulfuric acid is extracted with hexane. Prouty & Cromartie (1970) studied the recoveries of ^{14}C-DDT in each of the 5 major stages of a method for determining this compound in the tissues of quail; Soxhlet extraction with hexane for 6 h, of muscle, liver, heart or brain, after grinding with sodium sulfate gave recoveries of DDT-type compounds (as ^{14}C-activity) of 93–105%. Jonczyk (1970) used hexane to extract DDT-type compounds from blood and reported recoveries of 67–91%. Acetone was used as the extracting solvent for DDT-type compounds in the adipose tissue and brain of partridges (Jonczyk et al., 1970). Wood's method, using dimethyl sulfoxide, was used by Stec & Juszkiewicz (1972), and was found to give results for DDT-type compounds that compared favourably with other methods of analysis of animal tissue, eggs, and milk.

All methods of extracting DDT also result in the removal of lipids, if they are present. Regardless of the method of extraction, the results of most analyses have been reported in terms of fresh or wet weight of samples no matter whether their lipid content was extremely low (water and urine), low (soils and most vegetables), intermediate (milk and many tissues), or high (adipose tissue). In some instances, samples such as adipose tissue, milk, and, to a lesser degree, other animal tissues known to contain lipids have been reported in terms of the concentration of pesticide in extractable lipid. Because DDT and DDE are known to have a marked affinity for neutral fat, it was originally supposed that reporting in terms of lipid would reduce variability within any set of samples by excluding the influence of connective

(and sometimes lymphatic) tissue, which forms a part of each sample. It appears that variation, as measured by the coefficient of variation, may not be reduced by this kind of reporting (Casarett et al., 1968). However, there may be other reasons for reporting on a lipid basis and it is absolutely essential that the method of reporting be specified.

2.2.5 Clean-up procedures

The extraction procedures remove not only the DDT-type compounds from the samples analysed but also coextractives to a greater or lesser degree according to the type of sample. A number of procedures have been developed that reduce the amounts of the coextractives relative to that of DDT-type compounds. If interest is confined solely to DDT-type compounds, then their stability in the presence of concentrated sulfuric acid is a very useful clean-up procedure (Mattson et al., 1953; Czegledi-Janko & Cieleszky, 1968; Murphy, 1972). Usually, however, the concentrations of other compounds are also of interest and this procedure cannot be used if these compounds are not stable in concentrated sulfuric acid. Two general clean-up procedures, used in sequence, are appropriate in these circumstances, namely, liquid–liquid partition followed by liquid–solid partition. Liquid–liquid partition systems such as hexane/acetonitrile, hexane/dimethyl formamide, or hexane/dimethyl sulfoxide are the ones most commonly used. The liquid–solid partition systems generally consist of Florisil, silica gel, or alumina as the solid phase, and hexane or mixtures of hexane and various proportions of a polar solvent (e.g., diethyl ether) as the mobile phase. Separation of DDT-type compounds from triglycerides in fat-containing tissues is achieved with considerable efficiency by liquid–liquid partitions, the hexane/dimethyl formamide or hexane/dimethyl sulfoxide systems being generally more efficient than hexane/acetonitrile. Separation of DDT-type compounds from other organochlorine compounds (e.g., aldrin, dieldrin, polychlorinated aromatics) or from steroids is not very efficient using liquid–liquid systems, and the liquid–solid partition systems should be used in these cases. Separation of DDT-type compounds from polychlorinated biphenyls is particularly difficult and, as some of these compounds have similar liquid chromatographic retention times to those of the various DDT-type compounds, the analysis of samples containing both classes of compounds requires considerable care. Detailed clean-up procedures are described by de Faubert Maunder et al. (1964), Cieleszky et al. (1970), Anon. (1974), Thompson (1974), and by Horwitz (1975).

The separation of DDT-type compounds from polychlorinated biphenyl, by liquid–solid (silical gel) partition is discussed by Armour & Burke (1970), Snyder & Reinert (1971), and Masumoto (1972); the last-

mentioned investigator concluded that a number of factors required careful control if satisfactory separation of DDT-type compounds from polychlorinated biphenyls (PCBs) were to be achieved, in particular, the degree of activation of the silicic acid (irregular distribution of water molecules onto the silicic acid particles was also probably important). He found that separation of *p,p'*-DDE from four Arochlors was incomplete. A collaborative study of the separation of DDT-type compounds from PCBs using the Armour & Burke silicic acid column procedure has been reported (Sawyer, 1973).

Florisil and coconut charcoal have also been investigated for the separation of PCBs from DDT-related compounds (Reynolds, 1969; Benvenue & Ogata, 1970; and Stijve & Cardinale, 1974). Another procedure that has been developed for the separation of DDT-PCB mixtures is the oxidation of *p,p'*-DDE to *p,p'*-dichlorobenzophenone (Miles, 1972). A method for the determination of *p,p'*-DDT and a particular PCB isomer that has similar retention time to that of *p,p'*-DDT is based on an empirical relation for p-values (Zelinski et al., 1973).

2.2.6 Quantitation

2.2.6.1 *Determination of DDT-type compounds*

Two techniques have played a major role in the quantification of DDT-type compounds, one is a colorimetric method, the other (now the most widely used) is gas–liquid chromatography with a halogen-sensitive detector.

The colorimetric procedure of Schechter-Haller is described in detail in the Handbook of the Deutsche Forschungsgemeinschaft (1969), and by Cieleszky et al. (1970) and Horwitz (1975).

Gas–liquid chromatography is essentially a further method of separation of compounds, and, although it is the most effective of the separation procedures (apart possibly from high pressure liquid–liquid chromatography) it must be realized that it has its limitations, particularly if used with a highly sensitive but nonselective detector such as the electron-capture detector. The basic principles of gas–liquid chromatography are described by Dal Nogare & Juvet (1962) and Yermakov (1972), for example, and need not be discussed here. However, attention is drawn to three aspects of the performance of a column in the gas–liquid chromatographic separation of DDT-type compounds from other compounds. First, the DDT-type compounds should not undergo any thermal degradation or other chemical change; second, the performance of the column should be assessed by the number of theoretical plates; and third, the performance of

the column as regards ability to separate *p,p'*-DDT and related compounds from other compounds that have similar retention times for a particular liquid phase must be investigated. Transport of *p,p'*-DDT and analogues through a column without chemical change is dependent upon the absence of reactive centres in the column. This can be attained by using an inactive solid support (by coating the reactive sites with a silane, if necessary) and ensuring that the surface of the solid support is completely covered by an inert liquid phase. The performance of a column should be checked at regular intervals; the US EPA Manual (Thompson, 1974) suggests a mixture containing five DDT-type compounds plus eight other organochlorine insecticides for this purpose. Change in peak shape (i.e., departure from symmetrical peaks) should always be regarded as a warning sign. In the case of serious doubt about the stability of *p,p'*-DDT or an analogue (which may manifest itself as a change in the retention time relative to that of aldrin or dieldrin for example), it is suggested that a fraction collection technique be used and the identity of the component leaving the column at a particular retention time with that injected confirmed.

Methods of calculating the number of theoretical plates and of separation factors are given in standard texts. According to the US EPA Manual, a column of 2 m length should have about 3000 theoretical plates. The factors that control the performance of gas–liquid chromatographic columns are discussed in detail by Scott (1970). Convenient summaries of the retention times (absolute or relative) of DDT-type compounds, together with those of other pesticides, using various column conditions have been published (Yermakov, 1972; Zweig & Sherma, 1972). Retention times of 51 pesticides relative to that of aldrin using six stationary phases at three temperatures with an electron-capture detector have been reported by Thompson et al. (1975); estimates of the relative retention times at other temperatures were derived from the relationship between relative retention time and temperature for each liquid phase. The relative times of DDT-type compounds and other pesticides on eight stationary phases were determined by Thompson et al. (1969a). Nonpesticidal organochlorine compounds that have retention times similar to those of DDT-related compounds include the polychlorinated biphenyls and polychloronaphthalenes.

Examples of the close similarity between the relative retention times of DDT-related compounds and those of various PCB isomers have been published (Bagley et al., 1970; Richardson et al., 1971; Stijve & Cardinale, 1974). Goerlitz & Law (1972) demonstrated that there are also similarities between the relative retention times of DDT-type compounds and those of various isomers of polychlorinated naphthalenes. Examples of similarities between the relative retention times of DDT and its analogues and various components in polychlorinated biphenyls and polychlorinated naphthalenes

was also reported by Griffith & Blanke (1974). Problems arise in the case of mixtures of toxaphene and DDT-related compounds (Cahill et al., 1970).

The effects of severe infection loading on column performance, peak configuration, and conversion of *p,p'*-DDT into *p,p'*-TDE, were studied by Thompson et al. (1969b). These investigators found that the columns they used could be maintained and restored to full or nearly full performance capacity by daily changing of the glass injection port insert and the glass-wool plug at the column inlet.

Two different types of detection systems are most frequently used for the quantification of *p,p'*-DDT and analogues after elution from gas–liquid chromatographic columns, namely, electrochemical detectors and electron-capture detectors. Two types of electrochemical detector have been developed, the microcoulometric detector and the microelectrolytic conductivity detector, that are considerably more sensitive to *p,p'*-DDT and its analogues than the original electrochemical detectors. Giuffrida & Ives (1969) described modifications and improvements in microcoulometric gas chromatography and, in the case of DDT-type compounds in carrots, they obtained responses from their microcoulometer that were approximately one-fifth of those given by a particular electron-capture detector; Griffith & Blanke (1974) described a microcoulometric method for the determination of *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE in blood. According to Dolan & Hall (1973), the microelectrolytic conductivity detector can be used for the selective determination of organochlorine pesticides in the presence of polychlorinated biphenyls. However, the relative selectivity in regard to *p,p'*-DDE does not appear to be as great as that for other organochlorine insecticides.

The electron-capture detector produces an extremely sensitive response to organochlorine compounds, but its response is not, unfortunately, very selective and many other classes of compounds have electron affinity in the vapour phase. A general review of the principles and characteristics of the electron-capture detector has been published by Pellizari (1974). The detector may be used under direct current or pulse sampling conditions; anomalous responses obtained in the direct current mode of operation are not present under pulse sampling conditions (Lovelock, 1963). A major limitation of the electron-capture detector is that its response is linear over a very limited range only, but a new mode of operation in which the linearity extends over about four orders of magnitude of response has been described by Maggs et al. (1971). For this the detector current is held constant while the frequency of the applied pulses is varied. The response of electron-capture detectors is liable to change significantly during use, and these detectors should be recalibrated at regular intervals, preferably at least once per day with single standard injections at frequent intervals between

injections of extracts from the samples under investigation. It is of interest that Mendoza (1971) reported a significant difference in the response of a gas–liquid chromatograph to *p,p'*-DDT when injections were made at fast and slow rates.

The combination of mass-spectrometry with gas–liquid chromatography was mentioned in section 2.2.4 as a means of confirming the identity of residues of DDT-type compounds. This combination of instruments can also be used to quantify the amounts present. The total ion current corresponding to mass fragments of appropriate mass number (m/e; in the case of *p,p'*-DDE, 316, 318, 246 and 248) is measured and the amount present is calculated from the calibration of the instrument, preferably by an internal standard. Palmer & Kolmodin-Hedman (1972) determined the concentration of *p,p'*-DDE in human plasma by mass-fragmentography using the ion current corresponding to m/e 216 and 218; the results showed an excellent agreement with the values obtained using an electron-capture detector.

Semiquantitative methods of the determination of *p,p'*-DDT and its analogues include paper chromatography and thin-layer chromatography; these methods, which are more appropriately used for the confirmation of identity of residues, are described by McKinley (1963) and Sherma (1973) respectively. Bishara et al. (1972a) have given the tlc R_f values for *p,p'*-DDT and 14 related compounds using 33 solvent systems.

2.2.6.2 *Determination of p,p'-DDA in urine*

A major metabolite of *p,p'*-DDT, excreted in the urine, is bis-(4-chlorophenyl)acetic acid (*p,p'*-DDA). A method has been developed by Cranmer et al. (1969), based upon the extraction of *p,p'*-DDA from acidified urine, conversion into the methyl ester, Florisil column clean-up, and gas–liquid chromatographic analysis of the ester. A detailed outline of this procedure is given by Thompson (1974). The sensitivity of response of the methyl ester is not high, the limit of detection being about 2 ng. Cranmer & Copeland (1973) used the 2-chloroethanol ester, the response of an electron-capture detector being about 3.7 times greater than that of the methylester (i.e., limit of detection about 0.5 ng). An advantage of this ester is that it is well separated from *p,p'*-DDT, whereas the methyl ester has a retention time similar to that of *p,p'*-DDE. The retention time of the pentafluorobenzyl ester compound is about twice that of *p,p'*-DDT and the response of an electron-capture detector is considerably greater than that for the 2-chloroethanol derivative (Johnson, 1973).

2.2.6.3 *Method of reporting results*

Francis Galton (1879) pointed out that many vital effects are distributed logarithmically; his paper was followed by a technical one (McAlister,

1879) presenting the mathematics. Apparently Robinson & Hunter (1966) were first to point out that this principle applies to the storage of pesticides so that the geometric mean is a more appropriate parameter than the arithmetic mean for expressing insecticide content. In recent years, increasing use has been made of the geometric mean as recorded by footnotes in Table 9. Few of the arithmetic means that have been reported are so much in error that they should be discarded. As Galton (1879) noted, the difference between the arithmetic and geometric mean is small if the range of the values averaged is narrow.

2.2.7 Validation of analytical methods for DDT-type compounds

Ideally, an analytical method should be accurate and highly precise. A study of the accuracy and precision of an analytical method must be made in order to assess the relationship between the actual amount present and the results obtained in practice; such a study may be called the validation of the procedure. There are two major types of validation procedure. The first type requires the use of radio-labelled molecules of *p,p'*-DDT, e.g., ^{14}C *p,p'*-DDT. Prouty & Cromartie (1970) determined the ^{14}C-activity in muscle, liver, heart, and brain of quail that had been given ^{14}C-labelled *p,p'*-DDT. Four of the steps in the analytical procedure were studied and it was concluded that the major discrepancies occurred during the elution and concentration steps from Florisil columns and from zones of thin-layer chromatography plates. Chiba & Morley (1968) used ^{14}C-labelled *p,p'*-DDT in a study of soil analysis.

The other validation procedure, which has been studied more intensively, involves the comparison of the results of analyses with the values for samples fortified with accurately known amounts of *p,p'*-DDT or analogues. There are several variations of this validation procedure, all comparing the fortified (nominal) value with the results of different methods in the same laboratory, with the results of a specified method carried out in different laboratories, or with the results of different methods in different laboratories.

Versino et al. (1971) compared 8 clean-up procedures for test mixtures containing 10 pesticides. Two extracts, from apples and lettuce, were fortified (spiked) with *p,p'*-DDT at 0.2 mg/litre, *p,p'*-TDE at 0.1 mg/litre, and *o,p'*-DDT at 0.1 mg/litre plus dieldrin and 7 organophosphate insecticides. The recoveries for the 8 column clean-up procedures ranged from 89–100% for *p,p'*-DDT, 85–101% for *p,p'*-TDE, and 95–100% for *o,p'*-DDT. It should be noted that this investigation did not include a study of extraction efficiency and that the extracts contained only small amounts of lipids. In studies by Smart et al. (1974), samples were analysed of milk, butter, cheese, eggs, apples, potatoes, carrots, and cabbages, fortified with *p,p'*-

DDT, *p,p'*-TDE, and *p,p'*-DDE (and 5 other organochlorine insecticides) at concentrations corresponding to those suggested as limits by the Codex Alimentarius Commission. Five replicate analyses of each sample were made, using up to 4 procedures, the final step of each analytical procedure being gas–liquid chromatography/electron-capture detection, with 3 different stationary phases. These workers concluded that there were no gross discrepancies in their results. However, they did not use the concept of "total error" (see above) in the discussion of their results, and the total errors for the determination of the 3 DDT-type compounds, calculated from their results, were in the range of 12% to 95%.

Results have been reported (Carr, 1970) of a collaborative study by 10 laboratories of the AOAC method for the determination of 4 DDT-type compounds in samples of butterfat fortified by the addition of these 4 compounds at 2 different concentrations. The mean recoveries varied from 86–108%, and the coefficients of variation between laboratories were in the range 7–28%. The total errors for the 2 levels of fortification respectively were: *p,p'*-DDT, 22 and 14%; *p,p'*-TDE, 17 and 30%; *p,p'*-DDE, 17 and 36%; and *o,p'*-DDT, 30 and 43%.

Carr (1971) gives the results of a collaborative study by 8 laboratories of the analysis of fish samples fortified (2 concentrations) with *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE, and 3 other organochlorine insecticides. On the basis of a ranking technique (Youden & Steiner, 1975), the results of 2 laboratories (Nos. 2 and 8) for the 6 compounds were rejected as outliers. However, if the results for the 3 DDT-type compounds are considered, then laboratories 7 and 8 gave results that are outliers. The total errors at the 2 concentrations for the 6 laboratories not rejected by Carr were: *p,p'*-DDT, 15 and 36%; *p,p'*-TDE, 32 and 32%; *p,p'*-DDE, 26 and 39%.

Sawyer (1973) reported a collaborative study by 9 laboratories of samples of red salmon fortified with 3 DDT-type compounds and PCBs. The analyses were carried out without and with silicic acid column separation of PCBs from DDT-type compounds. The total errors without and with silicic acid separation, were 34% and 43% respectively for *p,p'*-DDT, 50 and 47% for *p,p'*-TDE and 35 and 36% for *p,p'*-DDE. Very similar results were obtained with another type of PCB/DDT mixture.

Samples of soil, fortified with 4 DDT-type compounds plus 6 other organochlorine insecticides were analysed in 7 laboratories (Woolson, 1974); the total errors (all collaborators) were: *p,p'*-DDT, 17–33%; *p,p'*-TDE, 19–37%, *p,p'*-DDE, 18–42%; *o,p'*-DDT, 28–56%.

Fifteen laboratories collaborated in a study of the determination of *p,p'*-DDE in eggs, and *p,p'*-DDT, *p,p'*-DDE and *o,p'*-DDT in kale (Finsterwader, 1976). Five laboratories analysed eggs by the AOAC method, and the total error was 21.1%; the total error for 10 laboratories using a modifi-

cation of the AOAC method was 30.4%. The results of one laboratory for analyses of kale were rejected as outliers, and the total errors for the other 14 laboratories were: *p,p'*-DDT, 19.7%; *p,p'*-DDE, 18.8%; *o,p'*-DDT, 17.4%.

An international collaborative programme on methods of analysis of organochlorine insecticides has been sponsored by the Organization for Economic Cooperation and Development (OECD), and the results of 2 studies have been published. In the first (Holden, 1970), a solution in hexane of 6 organochlorine compounds, 3 being DDT-related compounds, was analysed by 17 laboratories in 11 countries. The total errors for *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE were in the range 14–21%, the major component in this total error being the standard deviation of results from different laboratories, probably indicating errors of calibration. In the second study (Holden, 1973), a sample of corn oil fortified with 4 DDT-type compounds and 3 other organochlorine insecticides was analysed in 19 laboratories in 10 countries: the mean total error for the 4 DDT-type compounds (after excluding 3 outliers) was 35%.

Some of the results of this collaborative validation study are very satisfactory, but many of them, as judged by the criterion of total error, are unsatisfactory, even when outliers are rejected, and they illustrate the need for scrupulous care by analysts: the use of clean glassware, chemicals of established purity, and constant checking of the performance of liquid–solid partition columns and of instruments.

2.2.8 Analytical methods for the evaluation of the biochemical effects of *p,p'*-DDT and its analogues

The increased activity of enzymes, particularly in the hepatic endoplasmic reticulum, if the exposure to *p,p'*-DDT and its analogues is sufficiently great, has been recognized in recent years. Measurement of such changes in enzyme activity may be made by studying changes in the rates of metabolism of certain drugs (such as antipyrine), but methods that do not require the administration of a drug (and the collection of blood samples if the plasma half-life is used as the biochemical parameter) are preferable. Two methods, in which the concentration of either 6-β-hydroxycortisol or D-glucaric acid in the urine is measured have been developed. A procedure for the determination of 6-β-hydroxycortisol in urine has been described by Kuntzman et al. (1968); and one for D-glucaric acid by Marsh (1963) (see section 8.2.5.1).

These enzyme induction changes are not specific for DDT-type compounds.

3. SOURCES OF ENVIRONMENTAL POLLUTION

3.1 Discovery and Introduction

DDT does not occur naturally. It was first synthesized by Zeidler as reported in 1874. However, it was not put to any use until its insecticidal properties were discovered by Paul Müller in 1939. The Swiss patent was issued in 1942.

As an example of the speed with which DDT was developed and used, the first sample sent to the USA arrived there in September 1942. This sample was tested for effectiveness and safety. The results were so encouraging that manufacture was given high priority. At first, the entire production was used for the protection of troops against malaria, typhus, certain other vector-borne diseases, or against biting flies or other insects that are merely pests. As the supply increased, DDT was used in the USA for control of malaria in military areas, that is in the vicinity of military installations, ports, and transportation centres. As a result of this effort, mosquito transmission of malaria was brought to an end in the USA in 1953, even though military personnel and other infected persons from the tropics continued to reintroduce the disease extensively as late as 1972 and to a lesser degree thereafter.

3.2 Production and use

The revolution in the control of malaria and typhus among allied troops and among certain civilian populations during World War II was accomplished with relatively little DDT. Far greater amounts were required for the control of agricultural and forest pests and this became possible when the compound was released in the USA for commercial use on 31 August 1945. Civilian use in other countries became possible a little later, first, largely on the basis of importation and gradually on the basis of local manufacture. Unfortunately, there is apparently no record of world production of DDT. Production and use in the USA is shown in Table 2.

Quantities of DDT and related compounds used in or sold for agricultural purposes in 1970 were as follows (metric tonnes): Australia (about 1000); Austria (20.5); Botswana (2.0); Canada (287.0); Columbia (980.0); Czechoslovakia (270.0); Democratic Kampuchea (46.8); Egypt (3457.0); El Salvador (466.0); Federal Republic of Germany (152.0); Finland (6.1); Ghana (0.3); Guatemala (380.0); Hungary (20.6); Iceland (0.3); Israel (10.0); Italy (2178.0); Japan (401.0); Kuwait (0.2); Madagascar (176.0);

Ryukyu Islands (Japan) (0.3); Sri Lanka (16.6); Sudan (269.0); Upper Volta (1.5); and Uruguay (5.0) (FAO, 1972). These values total 10 146.2 metric tonnes. Thus, at least until very recently, the use of DDT was extensive on a worldwide basis but varied greatly from one country to another.

Table 2. Metric tonnes of DDT produced and used in the USA[a]

Year	Production	Use
1944	4 366	
1945	15 079	
1946	20 220	
1947	21 534	
1948	9 181	
1949	19 822	
1950	35 448	
1951	48 144	
1952	45 327	
1953	38 268	28 349
1954	44 088	20 465
1955	56 760	28 032
1956	62 441	34 194
1957	56 136	32 205
1958	65 920	30 255
1959	71 097	35 771
1960	74 471	31 818
1961	77 763	29 061
1962	75 764	30 502
1963	81 154	27 744
1964	56 113	22 925
1965	63 859	24 034
1966	64 115	20 685
1967	46 906	18 260
1968	63 231	14 848
1969	55 839	13 724
1970	26 860	11 316

[a] Based on data from US Tariff Commission (Hayes, 1975).

3.3 Changing Patterns of Use

Before 1945, all of the DDT produced in the USA for example, was used or allocated by the military services for various medical and public health uses. Early in 1945, it became available for rather extensive experimental work in agriculture, and it was commercially available in limited quantities early in the autumn of the same year (US Department of Agriculture, 1945a,b). The results were so spectacular that use increased until 1959. In response to a demand for exports, production continued to increase to about 1963. Even before then some restrictions were placed on its use, mainly to minimize residues in food and in the feed of animals that produce milk and meat. Among the first of these restrictions was that on its use on dairy cattle or in dairy barns (USDA, 1949). Another important factor reducing the use

of DDT was the increasing resistance of pests. One of the first species to be affected was the house-fly; because of its abundance and widespread distribution, its resistance was bound to be noticed by the public, generally. Although many pests of public health importance became resistant to DDT in some or all of their range, resistance among vectors of malaria was less marked. Because malaria control constitutes such a large segment of vector control, the use of DDT for vector control has tended to remain stable, while its use in agriculture has continued to decline, especially in temperate climates.

The ban on the use of DDT and certain other organochlorine insecticides in Sweden from 1 January 1970 was based on a number of ecological considerations.

Government agencies of some other countries attempted to justify severe restrictions on the use of DDT by alleging that it was a threat to human health. This was in response to ecologists who considered that the widespread occurrence of DDT in the environment was inherently bad and was the direct cause of injury to certain fish and birds. However, this did not prevent the same agencies from making a proviso that DDT might be used, if needed, to combat any future threat from vector-borne disease within their boundaries.

Of course, before the restrictions were put into effect, it had already been possible to eradicate malaria in the USA and Italy, for example, and to control for the first time an epidemic of typhus in Italy and Germany (Simmons, 1959). Apparently, there have never been accurate figures on the proportion of the compound used for agriculture and for public health even in those countries that have recorded their total use of the compound.

As the situation now stands, DDT is still used extensively, both in agriculture and for vector control, in some tropical countries. Apparently, information is not available on how much of the agricultural use involves food protection or how much loss of food production would result if the use of DDT were discontinued. The picture with malaria control is clear. Substitution of malathion or propoxur for DDT would increase the cost of malaria control by approximately 3.4- and 8.5-fold, respectively, and this increase could not be supported. If DDT were not used, vast populations in the malarious areas of the world would be condemned to the frightening ravages of endemic and epidemic malaria (WHO, 1971).

4. ENVIRONMENTAL TRANSPORT AND DISTRIBUTION

Because DDT has been sprayed on people, domestic animals, buildings, agricultural crops, and forests, it is not surprising that it is now distributed widely in the environment. During the application of any spray or dust to a field, it is often possible to observe drift of the particulate material. This is especially true if the application is made by aircraft or by ground equipment that shoots the spray to the top of orchard trees. If the application is made to forests, it is very likely that at least part of the spray will fall directly on streams or lakes. Following application, redistribution is inevitable. As discussed more fully in the following sections, some DDT in soil enters the air by evaporation or on wind-blown dust. Even if watercourses are avoided initially, some insecticide will be washed into them by rains, mainly in conjunction with soil particles.

4.1 Local Drift in Air

The fact that there is drip is implicit from measurements of DDT on surfaces just after application. Wilson et al. (1946b) recovered only 7.5% of the nominal dose from plants; this proportion is typical but ignores material that may have fallen between plants or between leaves and come to rest on the ground. Of greater interest are measurements of total recovery made by means of absorbent targets laid out in advance. As might be expected, recovery of aerosols is less than recovery of sprays when other conditions of application are similar. Only 12.5% was recovered at the centre of swaths following application of a thermal aerosol by aircraft and recovery decreased at increasing distances from the centre (Hess & Keener, 1947). In another study, recovery was 8% for a thermal aerosol and 46% for a spray (Scudder & Tarzwell, 1950). In a different situation, the average seasonal recovery for DDT applied as a thermal aerosol ranged from 10 to 12%, while that for spray ranged from 56 to 76% (Tarzwell, 1950). However, somewhat lower recoveries of DDT sprays have frequently been reported: 30% (Hoffman & Merkel, 1948), 39% (Hoffman & Surber, 1948), and 27% (Surber & Friddle, 1949).

Most DDT particles that miss the target for which they are intended would be expected to fall in the general area. Studies of residues in soil and in wildlife indicate that this is true. The logarithm of the concentration of DDT-related compounds in soil samples collected in a desert area downwind from an area of intensive agriculture showed an almost straight line inverse relationship to the logarithm of the distance from the source. The soil levels

were about 1 mg/kg at 10 m and about 0.001 mg/kg at 100 000 m from the point of application. The concentrations of DDT in the tissues of wildlife were proportional to its concentrations in the soils of their habitats (Laubscher et al., 1971). Where cultivated fields to which DDT has been applied for years are interspersed among pastures and other fields where DDT is not used, the uncultivated soils contain only a little less DDT than the cultivated ones. In one study, it was found that most of the DDT was in the top 2.5 cm, but samples for comparison were taken to a depth of 7.5 cm; the average concentrations were 0.75 to 2.03 mg/kg in cultivated soil and 0.10 to 0.91 mg/kg in uncultivated soil (US Department of Agriculture, 1966). These concentrations may be compared (by mathematical calculation alone) to 1.0 mg/kg, the concentration resulting from mixing DDT into soil uniformly and with no loss whatever following application at the rate of 1.12 kg/ha, the standard specific gravity (1.47) of soil and a depth of 7.6 cm being assumed. The range of specific gravities for most agricultural soils is 0.4 to 2.0 and the corresponding depths yielding 1 mg/kg are 28 to 5.60 cm. It is of interest that the residues in cultivated soil were of the order of magnitude that would be expected from a single application, even though the average cumulative rate of application during the last 10 years (11.2 kg/ha) had been 10 times as great.

4.2 Distant Drift in Air

Dust bearing DDT at a concentration of 0.6 mg/kg has been observed about 1600 km from its area of origin. Other pesticides were also present in this dust which had settled out on a recently rain-rinsed roof. The cloud of dust was so dense and unusual that its progress was reported in newspapers, as the storm that mobilized it in Texas carried it at least as far as Ohio, where the sample was collected (Cohen & Pinkerton, 1966).

Dust collected on the island of Barbados by means of nylon nets treated with 50% aqueous glycerine was assumed to have been blown from Africa, a distance of over 4850 km. Samples of the dust contained *p,p'*-DDT most frequently and in greatest concentration, but the concentration of all pesticides was only 0.001 to 0.164 mg/kg with an average of 0.041 mg/kg (Risebrough et al., 1968).

DDT evaporates from sprays and dusts at the time of application and at any time that dust bearing the compound is mobilized by the wind. Furthermore, DDT can be detected over treated fields for more than six months after application, and there is a concentration gradient from the soil upward (Willis et al., 1971). Under these circumstances, it is obvious that DDT is transported in the form of a vapour as well as by means of

dust. However, if samples are collected where no application of DDT has been made, identification of the origin of the vapour or measurement of the distance it may have travelled is even more difficult than with dust.

Evidence that DDT in one form or another has travelled great distances and is, in fact, worldwide in its distribution has been deduced from finding it in the rainwater of remote, nonagricultural places (Tarrant & Tatton, 1968) or in water melted from Antarctic snow (0.00004 mg/litre) (Peterle, 1969). In such remote places as Eskdalemuir in Scotland and Lerwick in the Shetland Islands, the average concentrations of *p,p'*-DDT in rainwater (0.000030 and 0.000046 mg/litre, respectively) were not greatly different from the averages (0.000018–0.000066 mg/litre) found in widely separated agricultural areas, suggesting that the compound is rather evenly distributed in the air (Tarrant & Tatton, 1968).

4.3 Distribution in Water

DDT has a strong tendency to adsorb on surfaces. Most DDT that enters water is already firmly attached to soil particles, and remains attached. It was shown very early that, if DDT does find its way into clear water, it is gradually lost by adsorption on surfaces (Carollo, 1945). The sediments in water tend to move downstream and eventually to enter estuaries. Of the various chlorinated hydrocarbon insecticides, DDT and its metabolites are the ones most commonly found, but the residues tend to be low (Butler, 1969). In fact, in an estuary associated with the Mississippi River, the levels of pesticides decreased strikingly from the early 1960s to the late 1960s (Rowe et al., 1971).

4.4 Bioaccumulation of DDT and Its Degradation in the Environment

Many studies of DDT and related compounds in the environment have focused on organisms and locations in which concentrations of DDT have been observed to increase. Concentration in living organisms may be the result of adsorption from water, of the filtering out of algae or detritus bearing the compound, or of biological magnification in the strict sense, that is progressive accumulation in different steps of a food chain. Although the mechanisms are poorly understood, observation has shown that residues do reach an equilibrium and sometimes decline. For example, where DDT was applied to cotton at a cumulative rate of 11.2 kg/ha so that a residue of over 10 mg/kg in the soil would be expected after many years of continual use, the actual residues ranged from 0.75 to 2.03 mg/kg (USDA, 1966). An

example of decreased residues was seen in the grebes of Clear Lake (Rudd & Herman, 1972).

Evidence is accumulating that the disintegration of DDT may be rapid in some situations. Under biologically active, anaerobic conditions as little as 1% of DDT remained after 12 weeks of incubation (Hill & McCarty, 1967; Guenzi & Beard, 1968). Probably more important is the disintegration of DDT under the influence of ultraviolet light. Hartley (1969) pointed out that much of any pesticide vapour escaping to 50 metres or more above the ground will ascend even higher by eddy diffusion and eventually reach the photochemically active ionosphere. The rapid destruction of DDT by ultraviolet light under laboratory conditions has been demonstrated (Mosier et al., 1969; Plimmer et al., 1970; Miller & Narang, 1970; Plimmer & Klingebiel, 1973; Crosby & Moilanen, 1977). Gab et al. (1975, 1977) offered evidence that DDT and DDE are converted to carbon dioxide and hydrochloric acid; the destruction was so complete that they characterized it as photomineralization. In spite of very real progress in understanding the fate of DDT in the environment (see Annex), much more work will be required before a quantitative balance can be measured between addition of the compound and its disintegration.

5. ENVIRONMENTAL EXPOSURE LEVELS

5.1 Exposure of the General Population

5.1.1 DDT in air

In spite of the generalization in section 4.2 that DDT is rather evenly mixed in the air, some increase in concentration may be noted in connexion with the time and place of application. The highest concentration of insecticide found in the air of communities with anti-mosquito fogging programmes was 0.0085 mg/m^3 (Tabor, 1966). Concentrations one or two orders of magnitude greater have been reported for several insecticides in the breathing zone of orchard spraymen, and values of 1.2–0.26 mg/m^3 have been found at distances of 500–5000 m from ground spraying (Belonožko et al., 1967). In six small communities in an agricultural area in the USA, DDT was found in concentrations ranging from 1×10^{-6} to 22×10^{-6} mg/m^3 (Tabor, 1966). Substantially higher values (0.002–0.05 mg/m^3) were reported for centres of population in the USSR (Belonožko & Kučak, 1974). In an urban location in a generally nonagricultural area of the USA, the highest concentration found was 2.36×10^{-6} mg/m^3 (Antommaria et al., 1965). The combined concentrations of DDT, dieldrin, and lindane in the Munich area of the Federal Republic of Germany in 1971 were even lower, only exceptionally rising as high as 1×10^{-6} mg/m^3 (Weil et al., 1973).

With few exceptions, the highest average concentration of DDT in air to which workers are exposed regularly (7.1 mg/m^3) is that associated with spraying the interior of houses (Wolfe et al., 1959). However, concentrations ranging from 2 to 104 mg/m^3 have been reported in places where DDT dust was prepared and packed (Medved' et al., 1975).

5.1.2 DDT in water

Under agricultural conditions, the concentration of DDT in water may be high. For example, 0.01 mg/litre was found in the runoff from melting snow from fields where sugar-beets had been grown (Medved' et al., 1975).

The highest level at which DDT has been found in rainwater in an urban area during a period of a month is 0.0004 mg/litre (Abbott et al., 1965). The highest concentration reported in potable water (0.02 mg/litre) occurred some years ago (Middleton & Lichtenberg, 1960). In a much more comprehensive study made a few years later, the highest concentration of any insecticide was found (0.00012 mg/litre), but this was dieldrin and not

DDT (Weaver et al., 1965). Many samples did not contain detectable insecticide of any kind. A study of surface waters in the USA during the years 1964–1968 indicated that the residues reached a peak in 1966 and then dropped sharply in 1967 and 1968, in spite of improved analytical methods. The highest value for a DDT-related compound in those years was 0.00084 mg/litre (Lichtenberg et al., 1970). By 1971, the concentration in the Federal Republic of Germany was even lower, averaging 0.00001 mg/litre and never going as high as 0.001 mg/litre (Weil et al., 1973). The average values for total DDT in drinking water in Czechoslovakia were 0.000011 and 0.000015 mg/litre in 1972 and 1973, respectively (Hruška & Kociánová, 1975). DDT was not detected (<0.0000000166 mg/litre) in tap water in a recent survey carried out in Ottawa, Canada (McNeil et al., 1977).

5.1.3 DDT in food

Residues of DDT were measured as early as 1945 (before the compound was available commercially) on apples to which it had been applied experimentally for the control of the coddling moth (Harman, 1946). Apparently, the earliest effort to learn how much DDT the average man obtains from his daily food was that of Walker et al. (1954), who reported that the amount of insecticide in restaurant meals in Wenatchee, Washington, USA indicated an average intake of 0.184 and 0.102 mg/man per day for DDT and DDE, respectively. Soon, other studies revealed similar levels of DDT intake for persons who ate an ordinary range of foods but who lived in different parts of USA (Hayes et al., 1956; Durham et al., 1965b). Most of the DDT was in food of animal origin, and persons who abstained from eating meat but obtained the food they ate from regular, commercial sources received an average of only 0.041 and 0.027 mg/man per day of DDT and DDE, respectively (Haynes et al., 1958). The difference did not depend, however, on meat *per se*, for no DDT and only traces of DDE were found in the meat and other products obtained from Arctic wildlife that constituted much of the diet of Eskimos (Durham et al., 1961).

Following restrictions on the application of DDT to livestock, to their barns, and to the forage crops on which they fed, there was a gradual decrease in residues in animal products used as human foods. Restrictions on the use of DDT on crops eaten directly by man resulted in reduced residues in vegetable foods. Complete meals collected mainly from the same restaurants in Wenatchee and analysed in the same laboratory indicated that, by 1964, DDT intake was only 0.038 mg/man per day (Durham et al., 1965b) compared with 0.184 mg/man per day reported by Walker et al.,

years earlier. Thus, intake had been reduced to less than one-fourth. A further reduction to about one-eighth of the 1953–54 values was indicated by the nationwide study by the US Food and Drug Administration, usually called the Market Basket Survey. Intakes of DDT indicated by this study for the succeeding years 1965–70, were 0.031, 0.041, 0.026, 0.019, 0.016, and 0.015 mg/man per day, respectively (Duggan & Weatherwax, 1967; Duggan, 1968; Duggan & Lipscomb, 1969; Duggan & Corneliusen, 1972).

The widespread shipment of food in the USA tends to explain the fact that food residues are generally similar in different parts of the country. However, small differences do exist and may be accounted for by the fact that, on average, meat is not shipped as far as vegetables. Much of the feed for livestock is produced on the same farm or at least in the same area in which the animals are raised. Whatever the reason, the coefficients of correlation between latitude and human intake were –0.63 and –0.59 for the sampling periods 1966–67 and 1967–68, respectively (Hayes, 1975).

Early studies (Swackhamer, 1965) indicated that both the frequency and concentration of residues were slightly less in Canada than in the USA. More recent studies in Canada similar to the Market Basket Survey indicated total DDT dietary intakes of 0.018, 0.011, 0.011, 0.007 and 0.007 mg/man per day for the years 1969 to 1973, respectively (Smith, 1971; Smith et al., 1972, 1973, 1975).

The analysis of whole meals collected in south-east England during 1965 and 1966 gave results similar to those obtained during the same period in the USA; the calculated daily intakes in England were 0.030 and 0.025 mg/man per day for DDT and DDE, respectively (McGill & Robinson, 1968).

There may be considerable differences in intake in different parts of the same country. Hruška & Kociánová (1975) reported that the average intake of DDT plus DDE in 1972 was 0.002 mg/man per day in southern Bohemia and was 0.099 mg/man per day in Slovakia. Striking differences may exist between urban and rural areas (Almeida et al., 1975).

Values for total DDT-related compounds in regular food in the USSR are not available, but the separate values for DDT and DDE in daily diets from different regions shown in Table 3 suggest that the total may be higher than in the UK and USA and that the amount of DDE may exceed the amount of DDT. Both high total values and an unusually high proportion of DDE would be consistent with extremely high total values a few years earlier and recent discontinuation of the use of DDT. In fact, in the Soviet Union, DDT has been eliminated from the list of pesticides recommended for use in agriculture since 1970. During the period 1966–69, 0.8% of food samples contained residues as high as 5.1 mg/kg, and 4.2% contained residues over 1.0 mg/kg.

Although total intake of DDT from food has not been measured in some parts of the world, worldwide measurements of storage of DDT and its metabolites in human body fat indicate that the extremes of total exposure have varied by a factor of about 10, but that total exposure for most populations has varied by a factor of no more than 3 (see Table 9).

It is important to note that local practice may result in high residues in the food of one or more families even though residues are low in commercially produced food available in the same area. Thus Durham et al. (1965b) reported that the average DDT intake from household meals in Wenatchee was 0.314 mg/man per day at the same time that restaurant meals in that town contributed only 0.038 mg/man per day. The difference was largely the result of very high residues in some eggs eaten by local families; the chickens foraged near orchards, which had been treated with DDT. The restaurants used commercially available eggs and not those produced locally. In a similar way, practices peculiar to one country may account for high residues in some of their food. For example, very high residues of DDT were reported in some samples of staple foods in India (Sharangapani & Pingale, 1954). Dale et al. (1965) suggested that the high levels of DDT that they found in some Indians might be the result of direct addition of the compound to staple food to prevent insect infestation, even though the practice did not have government approval.

5.1.4 Miscellaneous sources

It has been suggested that there is a positive correlation between the use of household insecticides and the concentration of DDT in house dust, on the one hand, and the storage of DDT in people on the other (Deichmann & Radomski, 1968; Radomski et al., 1968; Davies et al., 1969b, 1975; Edmundson et al., 1970b). However, another study of dust in 16 urban households, 4 farm households, and 8 households in which at least one member was a pesticide formulator, failed to reveal a statistically significant correlation between the levels of various pesticides in dust and in the serum of people living in the homes. There were striking individual examples of workers whose homes contained high concentrations of the compounds they used professionally and other examples in which there was circumstantial evidence relating household dust residues to body burden (Starr et al., 1974).

There can be no doubt that insecticides used in the household or introduced on the clothing of workers are important sources of intake of DDT in some instances. It is not clear whether the relevant absorption involves mainly the inhalation of dust, the contamination of food within the home, or even dermal absorption.

Table 3. Residues of DDT and DDE in daily cooked diets from different areas of the USSR during 1971/72[a]

Area	No. of daily diets examined	Range of residues of	
		DDT (mg/diet)[b]	DDE (mg/diet)[b]
North	121	0.00006–0.0103	0.0001–0.0093
West	191	traces–0.094	traces–0.039
South-east	62	traces–0.15	traces–0.330
South I	184	0.02–0.260	0.020–0.5
South II	51	traces–0.052	traces–0.132

[a] From: Medved' et al. (1975).
[b] Equivalent to mg/man per day.

5.1.5 Relative importance of different sources

It has been estimated (Campbell et al., 1965) that over 90% of the DDT stored in the general population is derived from food. About 1965, intake in the USA was approximately 0.04 mg/man per day from food, less than 0.000046 mg/man per day from water, less than 0.00006 mg/man per day from urban air and less than 0.0005 mg/man per day from air in small agricultural communities. The reason for the qualification "less than" is that the intakes were calculated from the highest concentrations reported in drinking-water and air because no average values were established.

Other investigators (Durham et al., 1965b; Morgan & Roan, 1970; Medved' et al., 1975) concluded independently that ordinary food is the major source of DDT and related compounds for the general population. Intake from ordinary food is a base to which other kinds of intake—including that from exceptional food—may be added. An example of such an addition—eating eggs from chickens allowed to run loose in DDT-treated orchards has already been discussed in section 5.1.3.

5.2 Exposure of Infants and Young Children

Babies tend to be born with slightly lower blood levels of DDT than are found in their mothers (O'Leary et al., 1970b; Schvartsman et al., 1974, see also section 6.2.2). This simply indicates that the placenta excludes some but not all of the DDT available to it. During the first 10 or 15 years of life, DDT storage levels rise to the adult population level (Hayes et al., 1958; Hunter et al., 1963; Wassermann et al., 1965, 1967; Robinson et al., 1965; Davies et al., 1968, 1969a; Watson et al., 1970).

It has been known for a long time that human milk may contain a higher concentration of DDT than cow's milk in the same country (Egan et al.,

1965; Quinby et al., 1965b; Ritcey et al., 1972; Olszyna-Marzys et al., 1973). So far, there is no evidence that this small difference is of any significance for breast-fed compared with bottle-fed babies. This is even true in places where the concentration of DDT in human milk is comparatively high (see section 6.3.1.4).

On the average, the incidence and levels of residues in commercially prepared food for babies in the USA are lower than those in raw agricultural products, other processed foods, or samples examined in the Market Basket Survey (Lipscomb, 1968).

The only DDT exposures of infants that are known to have injured them in any way are those involving direct access to formulations that they ate or drank. Such tragic accidents often involved formulations transferred to unlabelled food or beverage containers. Frequently, the container was left where a child could reach it easily. Occasionally, formulations have been stored with food or have even been handed to a child as food, or "empty" containers that still contain enough formulation to kill a child have been carelessly discarded (Hayes, 1975, 1976a).

5.3 Occupational Exposure

In general, annual exposure to DDT is greatest among manufacturers and formulators, moderate, among those applying it for agricultural purposes, less among the general population, and least among special groups whose location or practices minimize their exposure. However, for brief intervals the exposure during agricultural application may exceed anything that good industrial practice permits. This distinction is of great importance in connexion with the more toxic organic phosphorus compounds, a single heavy exposure to which may result in poisoning, but is of no known importance in connexion with DDT, the acute toxicity of which is much less. However DDT has a greater tendency to storage in the body.

Occupational exposure to DDT is reflected quantitatively by the concentration of DDT and DDE in blood and fat and by the concentration of DDA in urine. These aspects of occupational exposure are considered later. This section outlines measurements that have been made of the actual degree of exposure under different circumstances.

The most striking result is that the occupational exposure through areas of skin that are frequently unclothed (face, hands, forearms, neck, and "V" of chest) is far greater than total respiratory exposure. Results for DDT are shown in Table 4 (based on measurements made by methods described by Durham & Wolfe (1962, 1963)). If a workman has bare feet or legs or does

Table 4. Measured respiratory and dermal exposure of workers to DDT under actual conditions of work

Activity	Respiratory (mg/kg)	Dermal (mg/kg)	Reference
Indoor house spraying	3.4[a]	1755	Wolfe et al., 1959
Outdoor house spraying	0.11	243	Wolfe et al., 1959
Spraying forests	4–92[b]	212[b]	Wassermann et al., 1960

[a] Measurement by respirator pad technique.
[b] Calculated from values given in the original paper.

not wear a shirt, the contrast between respiratory and dermal exposure will be even greater than that shown in Table 4 which is based on "standard" clothing.

It has been reported that DDT and a number of other pesticides persist, for days or even years after last use, on the hands of workers exposed to them and that at least a part of the material can be removed for analysis by rinsing the hands in hexane. Evidence that the residues represented unabsorbed pesticides and not excretion of stored material included the fact that no correlation was found between residues on the hands and in the sera of individuals and that no residues could be found on the hands of a farmer who used rubberized gloves while working with pesticides and who washed afterwards with strong cleansing agents (Kazen et al., 1974). The fact that urinary excretion of DDA may be increased for several days after a single occupational exposure to DDT (Wolfe et al., 1970) is evidence that absorption continues for a week or so but not for longer periods. Furthermore, Wolfe et al. (1970) found that the rate of excretion, even when detectable, was small compared to the excretion of parathion metabolite in workers exposed simultaneously to DDT and parathion at a ratio of 1.0 to 0.25. This emphasizes the minimal dermal absorption of DDT.

6. METABOLISM OF DDT

6.1 Uptake

6.1.1 Uptake by inhalation

Most DDT dust is of such large particle size (⩾250 μm) that any that is inhaled is deposited in the upper respiratory tract and is eventually swallowed (Hayes, 1975). Toxicity data indicate that respiratory exposure is of no special importance.

6.1.2 Uptake from the gastrointestinal tract

A review of the early literature indicates that absorption of DDT from the gastrointestinal tract is slow. Whereas intravenous injection at the rate of 50 mg/kg produces convulsions in rats in 20 min, convulsions occur only after 2 h when DDT is administered orally at two or more times the LD_{50} value. The onset of convulsions is delayed for about 6 h when DDT is given to rats orally at approximately the LD_{50} value (Dale et al., 1963).

Early studies based on toxicity indicated that DDT, dissolved in animal or vegetable fats, was absorbed from the gastrointestinal tract about 1.5 to 10 times more effectively than undissolved DDT. There was also evidence that large doses of the compound in the gastrointestinal tract were poorly absorbed from nonabsorbable solvent (Hayes, 1959). However, in connexion with small repeated doses, the presence or kind of solvent made little difference; apparently the occurrence of bile in the intestine and the presence of some fat in the diet were sufficient to promote absorption of the compound. At high dosage levels, less ^{14}C-DDT was absorbed and stored in organs and a higher proportion was excreted in the faeces following oral administration than after intraperitoneal administration (40% versus 0.9%) (Bishara et al., 1972b).

Rothe et al. (1957) reported that after giving radioactive DDT to rats by stomach tube, 41–57% of it was recorded in lymph drained from the animal by means of a cannula. Less than 0.1% of the activity was found in the urine, 7.4–37.1% was found in the faeces or in the intestinal contents when the animals were killed, and 19%–67% of the activity was found in the carcass. The total dose accounted for analytically varied from 89% to 118%, thus recovery was complete within the accuracy of the method. Of the administered DDT not found in faeces and intestinal contents, 47%–65% was found in the lymph. The animals that withstood the operation best had peak lymph flows of nearly 6 ml/h. In these animals, DDT was absorbed at

rates as high as 381 μg/h; the rate of absorption reached a maximum within 2–3 h of incubation and was markedly reduced by the fourth hour. Fifty per cent of the DDT-derived material found in the lymph was absorbed in the first 2.5–7 h, and 95% was absorbed within 18 h. Because the lymphatic duct in the rat is not a single vessel, Rothe et al. (1957) were unable to exclude the possibility that some, or all, of the DDT that they later recovered from the carcasses of their animals had been transported to the general circulation by collateral lymph vessels rather than by the hepatoportal system. Thus, at least half, and perhaps all absorption of DDT is by way of the lymph. However, in studies on rats by Heath & Vandekor (1964), only a small proportion of dieldrin was absorbed by the lymph. The reason for the marked difference in the absorption of those organochlorine insecticides is unknown.

6.1.3 Uptake from the skin

Undissolved DDT is so poorly absorbed through the skin that its toxicity by this route is difficult to measure. Even dissolved DDT is poorly absorbed by the skin as indicated by low toxicity (see Table 5).

Table 5. Acute oral and dermal LD_{50} of DDT for animals[a]

Species	Formulation	Oral (mg/kg)	Dermal (mg/kg)
Rat	Water suspension or powder	500–2500	1 000 000
	Oil solution	113–450	250–3000
Mouse	Water suspension or powder	300–1600	375 000
	Oil solution	100–800	250–500
Guineapig	Water suspension or powder	2000	1 500 000
	Oil solution	250–560	1000
Rabbit	Water suspension or powder	275	375 000
	Oil solution	300–1770	300–2820
Cat	Water suspension or powder		
	Oil solution	100–410	
Dog	Water suspension or powder		
	Oil solution	>300	

[a] From: Hayes (1959).

6.2 Distribution and Storage

6.2.1 Human studies

6.2.1.1 *Studies of volunteers*

In a study of volunteers who received technical DDT at rates of 0, 3.5, and 35 mg/man per day, the average intakes resulting from dosing and from traces of DDT in food were 0.0025, 0.05, and 0.5 mg/kg per day (Hayes et

al., 1956). The storage of DDT was proportional to dosage, but there was an unexplained difference in the storage of the *p,p'*-isomer and of technical DDT. For example, following dosing for 12 months, pure *p,p'*-DDT was stored in fat at an average concentration of 340 mg/kg, but the sum of isomers from technical material was stored at an average of only 234 mg/kg. The difference was statistically significant for the 3.5 mg/man per day dosages given for 3–6 and for 7–18 months. The difference was significant for the 35 mg/man per day doses after 7–18 months of dosing but not after only 3–6 months.

Men who ate *p,p'*-DDT showed a definite increase in the absolute amount of DDE stored. After 6 months at a dosage of 35 mg/man per day, 8 men showed an average concentration of DDE stored in fat of 33 mg/kg compared with 12 mg/kg for the same individuals at the beginning of the investigation. There was a further increase in DDE storage as exposure progressed. However, DDT was stored in so much greater concentration that the relative storage of DDE decreased sharply. Thus, after 6 months at a dosage of 35 mg/man per day, 8 men stored only 14% of their total DDT-derived material in the form of DDE compared with 65% for the same person at the beginning of the investigation.

The storage of DDE by men who ate technical DDT presented a different picture. Until 18 months after exposure, there was no clear evidence that these men stored any more DDE after exposure than they did before. However, at 18 months, the only 3 samples available showed DDE concentrations ranging from 28 to 85 mg/kg, all substantially above general population levels. Thus, both the total amount stored and the rate at which DDT converted to DDE served to distinguish the metabolism of *p,p'*-DDT and the sum of isomers present in technical DDT in man (Hayes et al., 1956). A more rapid excretion was demonstrated for *o,p'*-DDT by Morgan & Roan (1972).

In a second study (Hayes et al., 1971), volunteers received the same doses used in the first study. Again, storage of DDT was proportional to dosage. Although, in this instance also, the storage of technical DDT was less than that of *p,p'*-DDT, the difference was not statistically significant. The real but very gradual accumulation of DDE was confirmed.

A steady state of storage was approached later in the second study (18.8–21.5 months) than in the earlier one (about 12 months). However, although the second study was superior in that more men were studied for a longer period, it was inferior in that dosage was less regular. Because of this, it seems impossible to decide whether 12 months or 21.5 months is a more valid estimate of the time necessary for people to approach a steady state of storage when intake is uninterrupted and unvarying in amount. It is interesting that the storage levels eventually reached at the same dosage in

the 2 studies were statistically indistinguishable in most instances (see Table 6). In the one instance in which a statistical difference existed, the greater storage by men in the second study may have been explained by the fact that some of them inadvertently received higher doses than intended.

There was a slow decrease in the levels of fat-stored DDT after dosing ceased. The concentration remaining following 25.5 months of recovery was from 32% to 35% of the maximum stored for those who had received 35 mg/man per day but 66% for those who had received only 3.5 mg/man per day, indicating slower loss at lower storage levels (Hayes et al., 1971).

Morgan & Roan (1971) fed volunteers not only technical DDT but also *p,p'*-DDE and *p,p'*-TDE. They found that DDE was stored more tenaciously than the other compounds in man, the order being *p,p'*-DDE > *p,p'*-DDT > *o,p'*-DDT ⩾ *p,p'*-TDE. The slow metabolism of DDT to DDE was confirmed. It was noted that *p,p'*-DDT was lost from storage in adipose tissue much more slowly in man than in the monkey, dog, or rat.

Less than 18% of *p,p'*-DDT and *p,p'*-DDE is carried in human erythrocytes. In plasma of ordinary fat content, less than 1% of all DDT-related compounds is carried by the chylomicrons. Instead, these compounds are carried by proteins and are undetectable in plasma from which protein has been precipitated. Following ultracentrifuging, *p,p'*-DDT and *p,p'*-DDE are found mainly in the triglyceride-rich, low density, and very low density lipoproteins. Following continuous electrophoresis, these compounds are found mainly in association with plasma albumin and α-globulins (Morgan et al., 1972).

6.2.1.2 *Studies of occupationally exposed workers*

The highest reported storage of DDT and related compounds remains that of a healthy worker whose fat contained DDT and DDE (as DDT) at concentrations of 648 and 483 mg/kg, respectively (Hayes et al., 1956). Laws et al. (1967) reported considerably lower storage values among the most exposed persons in a DDT manufacturing plant (see Table 7).

Table 6. Storage of DDT in volunteers

Type of DDT	Added dosage (mg/man per day)	Concentration of DDT[a] First study[b] 11 months or more (mg/kg)	Second study[c] 21.5 months (mg/kg)	Significance of difference (*P*)
Technical	0	8–17 (12.5 ± 4.5)	16–30 (22.0 ± 2.9)	>0.1
	3.5	26–33 (23.8 ± 1.4)	59–76 (50.2 ± 5.6)	<0.025
	35	101–367 (234 ± 21.4)	105–619 (281 ± 79.5)	>0.4
Recrystallized	35	216–466 (340 ± 36.4)	129–659 (325 ± 62.2)	>0.2

[a] Range, mean, and standard error.
[b] Hayes et al., 1956.
[c] Hayes et al., 1971.

Table 7. Average concentration of the sum of the isomers of DDT and DDE in fat and serum and of DDA in the urine of workers engaged in the manufacture, formulation, or use of DDT

Tissue	No. of men	DDT (mg/kg)	DDE (mg/kg)	DDA (mg/kg)	Total as DDT (mg/kg)	Estimated exposure (mg/man per day)	Reference
fat	1	648	437		1131		Hayes et al., 1956
urine	10			0.57		14	Ortelee, 1958
urine	16			1.7		30	Ortelee, 1958
urine	13			2.9		42	Ortelee, 1958
fat	3	51	44		98	3.6	Laws et al., 1967
fat	12	74	50		130	6.2	Laws et al., 1967
fat	20	161	91		263	18	Laws et al., 1967
serum	3	0.2113	0.1968		0.5412	6.3	Laws et al., 1967
serum	12	0.1420	0.1454		0.3584	8.4	Laws et al., 1967
serum	20	0.3020	0.2719		0.7371	17.5	Laws et al., 1967
urine	3	0.0165	0.0203	0.41	0.5629		Laws et al., 1967
urine	12	0.0145	0.0222	0.6	0.7911		Laws et al., 1967
urine	20	0.0145	0.0271	1.27	1.6296		Laws et al., 1967
fat	18				5.2–45.2		Gracheva, 1969
urine	136			0.402			Perini & Ghezzo, 1970
urine	110			0.142			Perini & Ghezzo, 1970

Table 7—*continued*

urine	290[a]			0.061		Perini & Ghezzo, 1970
plasma	16	0.0513	0.0722		0.1321	Wassermann et al., 1970c
serum	4	⩽0.087	⩽0.072			Edmundson et al., 1970a
urine				0.080		Edmundson et al., 1970a
serum	18	0.573	0.506			Poland et al., 1970
serum	5	0.004[a]	0.021[a]			Clifford & Weil, 1972
serum	10	0.022	0.055			Clifford & Weil, 1972
serum	21	0.021[a]	0.013[a]			Keil et al., 1972
blood	44				0.761	WHO, 1973
blood	100				1.273	WHO, 1973
serum	21	0.300	0.379		0.681	Almeida et al., 1974
serum	25	0.225	0.308		0.504	Almeida et al., 1974
serum	18	0.345	0.257		0.602	Almeida et al., 1974
serum	56	0.004[a,b]	0.052[a,b]			Morgan & Roan, 1974
serum	32	0.002[b]	0.026[b]			Morgan & Roan, 1974
serum	32	0.004[b]	0.047[b]			Morgan & Roan, 1974
serum	32	0.009[b]	0.075[b]			Morgan & Roan, 1974
serum	31	0.052[b]	0.222[b]			Morgan & Roan, 1974
plasma	25				1.030	Rabello et al., 1975
plasma	8				0.240	Rabello et al., 1975
plasma	23				0.0389	Richardson et al., 1975

[a] Control group.
[b] Approximately equal groups arranged by degree of storage.

An important point evident from the table is that, whereas almost all investigations of workers are said to have been carried out on "heavily exposed" populations, some of the groups studied had absorbed little more DDT than is absorbed by the general population—especially the general population of some tropical countries.

A different situation is indicated in a report by Genina et al. (1969) who used a total chloride method to analyse samples of blood from controls and from persons with occupational exposure to DDT, polychloropinene, and HCH. Whereas the highest average concentration of total DDT-related material *per se* in the serum of a worker in the USA was 2.7 mg/litre (Laws et al., 1967), Genina et al. (1969) reported organochlorine compounds as high as 38.4 mg/litre in the blood of a pilot and values as high as 195 mg/litre in the blood of warehousemen. This concentration is about 20 times the highest value found by the same authors in their control group (see Table 8). The factor of 20 is not remarkable, but (especially in view of the fact that polychloropinene and HCH are excreted more readily than DDT and DDE) values as high as 0.2 mg/litre in the controls are unexpected.

Table 8. Percentage of workers with blood organochlorine compound content falling in certain ranges of concentration[a]

Subject group	Range of concentrations					
	0 (mg/litre)	0.2–0.9 (mg/litre)	1.0–3.0 (mg/litre)	4–9 (mg/litre)	10–50 (mg/litre)	50 (mg/litre)
Control group (47)	21.3	44.7	23.4	10.6	0	0
Pilots (134)						
group A	19.1	35.3	25.0	14.7	5.9	0
group B	22.9	27.1	40.6	7.4	2.0	0
Technicians (133)						
group A	26.2	16.4	39.3	8.2	9.9	0
group B	39.4	31.2	25.7	3.7	0	0
Agricultural workers (55)	13.0	43.5	31.0	7.0	2.0	3.5

[a] From: *Genina et al. (1969)*.
NB: Group A gives the results of investigations at the time of work, group B before work or a few months after termination. Agricultural workers were studied only during work.

The first evidence that a part of the DDT absorbed by man is metabolized to DDE was obtained from the analysis of fat from a DDT plant worker (Mattson et al., 1953).

6.2.1.3 *Studies of the general population*

DDT in fat. Table 9 summarizes the results of measurements of DDT and related materials in the body fat of people without occupational exposure. Several broad generalizations can be made from the table and from what is known about residues of DDT in food in different countries.

Table 9. Concentration of DDT-derived material in body fat of the general population

Country	Year	No. of samples	Method of analysis	DDT[a] (mg/kg)	DDE as DDT (mg/kg)	Total as DDT (mg/kg)	DDE as DDT (% of total)	Reference
North America								
Canada	1959–1960	62	colour	1.6	3.3	4.9	67	Read & McKinley, 1961
Canada	1966	47	GLC[b] and ELC	1.09	2.96	4.39	67	Brown, 1967
Canada	1967–1968	51	GLC	1.56	4.16	5.86	71	Kadis et al., 1970
Canada	1969					4.85		Ritcey et al., 1973
Canada			GLC			5.83		Brown & Chow, 1975
USA	1942	10	colour	ND[c]	ND[c]	ND[c]		Hayes et al., 1958
USA	1950	75	colour	5.3	—	5.3	—	Laug et al., 1951
USA	1955	49	colour	7.4	12.5	19.9	63	Hayes et al., 1956
USA	1954–1956	61	colour	4.9	6.8	11.7	58	Hayes et al., 1958
USA	1956	36	colour	5.5	10.1	15.6	65	Hayes et al., 1971
USA	1961–1962	130	colour	4.0	8.7	12.7	69	Quinby et al., 1965a
USA	1961–1962	28[d]	GLC	2.4	4.3	6.7	64	Dale & Quinby, 1963
USA	1962–1963	282	GLC	2.9	8.2	11.1	74	Hoffman et al., 1964
USA	1964	64	GLC	2.5	5.1	7.6	67	Zavon et al., 1965
USA	1964	25	GLC	2.3	8.0	10.3	77	Hayes et al., 1965
USA	1964–1965	18	GLC			9.0		Schafer & Campbell, 1966
USA	1964–1965	42	GLC	3.1	7.5	10.6	71	Radomski et al., 1968
USA	1962–1966	994	GLC	2.6	7.8	10.4	75	Hoffman et al., 1967
USA	1964–1965	12	GLC	3.79	7.7	11.5	67	Davies et al., 1965
USA	1965–1967	17	GLC		3.1	5.5	56	Davies et al., 1968
USA		90	GLC		6.1	8.4	73	Davies et al., 1968
USA		17	GLC		4.6	7.8	59	Davies et al., 1968
USA		35	GLC		12.0	16.7	72	Davies et al., 1968
USA	—	42	GLC	3.13[e]	7.43	10.56	70	Fiserova-Bergerova et al., 1967
USA	—	30[e]	GLC	1.33	5.17	6.51	79	Casarett et al., 1968
USA		29[f]	GLC	1.35	4.91	6.31	78	Casarett et al., 1968
USA		30[g]	GLC	1.16	4.99	6.17	81	Casarett et al., 1968
USA	1966–1968	70	GLC	1.54	5.15	6.69	77	Morgan & Roan, 1970
USA	1967	733	GLC	1.34	4.74	6.22	77	Yobs, 1969 (unpublished data)
USA	1968	3104	GLC	1.56	5.96	7.67	77	Yobs, 1969 (unpublished data)
USA	1967–1971	103	GLC	1.5	5.6	7.1	79	Warnick, 1972
USA	1970	200	GLC	1.9	8.0	9.9	81	Wyllie et al., 1972
USA	1969–1972	221				23.18		Burns, 1974
USA	1970	1412				7.87[h,i]		Kutz et al., 1974
South America								
Argentina	1967	37	GLC	5.5	6.5	13.2	—	Wassermann et al., 1968b
Brazil	1969–1970	38	GLC	1.4	2.7	4.1		Wassermann et al., 1972b
Venezuela	1964	38	GLC	2.9	7.4	10.3	72	Dale, 1971 (unpublished data)
Europe								
Austria						6.33		Pesendorfer et al., 1973
Belgium		20	GLC	1.2	2.1	3.3	64	Maes & Heyndrickx, 1966
Bulgaria		55	GLC	3.8	5.8	10.6[h]	57	Kaloyanova et al., 1972
Bulgaria	1971–1976	191	GLC			14.7	78	Rizov, 1977
Czechoslovakia	1963–1964	229	colour	5.5	4.1	9.6	43	Halacka et al., 1965
Denmark	1965	18	GLC	0.6	2.7	3.3	82	Weihe, 1966
Denmark	1972–1973	78	GLC		4.1	4.7	87	Kraul & Karloq, 1976
Finland	1972–1974	73	GLC			2.5		Hattula et al., 1976
France	1961	10	colour	1.7	3.5	5.2	67	Hayes et al., 1963
German Democratic Republic	1966–1967	100	GLC and TLC	3.7	9.47	13.1	71	Engst et al., 1967
German Democratic Republic		34[j]		1.8	5.1	6.9		Engst et al., 1970
Germany, Federal Republic of	1958–1959	60	colour	1.0	1.3	2.3	57	Maier-Bode, 1960
Germany, Federal Republic of	1970	20	GLC	1.1	2.5	3.6	69	Acker & Schulte, 1970
Germany, Federal Republic of						9.8		Acker & Schulte, 1971
Germany, Federal Republic of						4.24		Acker & Schulte, 1974
Germany, Federal Republic of						4.77		Acker & Schulte, 1974
Germany, Federal Republic of						5.42		Acker & Schulte, 1974
Germany, Federal Republic of						8.36		Acker & Schulte, 1974
Germany, Federal Republic of						7.8		Acker & Schulte, 1974
Hungary	1960	48	colour	5.7	6.0	12.4	48	Denés, 1962

Table 9—*continued*

Country	Year	No. of samples	Method of analysis	DDT[a] (mg/kg)	DDE as DDT (mg/kg)	Total as DDT (mg/kg)	DDE as DDT (% of total)	Reference
Italy	1965	9	GLC	1.8	3.2	5.0	63	Kanitz & Castello, 1966
Italy	1965–1966	18	GLC	2.58	8.28	10.86	76	Paccagnella et al., 1967
Italy	1966	22	GLC and TLC	4.69	10.69	15.48	68	Del Vecchio & Leoni, 1967
Italy	1970?	31	GLC	3.38	13.37	16.75	80	Prati & Del Dot, 1971
Italy	1970?	52	GLC	2.14[a]	8.46[a]	10.60[a]	80	Prati et al., 1972
Italy	1970?	33	GLC	0.84[a]	6.64[a]	7.48[a]	89	Prati et al., 1972
Netherlands	1964	20	colour	1.6	6.1	7.7	79	Wit, 1964
Netherlands	—	11	GLC	0.32	1.89	2.22	86	deVleiger et al., 1968
Norway		56	GLC			3.2	71	Bjerk, 1972
Poland	1965	72	colour			13.4		Bronisz et al., 1967
Poland		65	colour			23.5	62	Bronisz et al., 1969
Poland	1970	70	GLC			11.4		Juszkiewicz & Stec, 1971
Poland	1972	15	GLC			5.23	68	Bojanowska et al., 1973
Romania	1965	137	—	13.4	8.3	21.7	39	Aizicovici et al., 1968
Romania	1972–1973		GLC	0.68	1.73	2.41	71	Ciupe, 1976
Spain	1966	41	.....	6.5	9.2	15.7	59	Unpublished data cited by Wassermann et al., 1968
Switzerland						1.9–16.3		Zimmerli & Marek, 1973
United Kingdom	1961–1962	131	colour	—	—	2.2	—	Hunter et al., 1963
United Kingdom	1963–1964	66	GLC	1.1	2.2	3.3[b]	67	Egan et al., 1965
United Kingdom	1964	100	GLC	—	—	3.9[b]	—	Robinson et al., 1965
United Kingdom	1964	44	GLC	—	—	4.0[b]	—	Robinson & Hunter, 1966
United Kingdom	1965	101	GLC	1.13	1.72	2.85	60	Cassidy et al., 1967
United Kingdom	1965–1967	248	GLC	0.78	2.22	3.00	74	Abbott et al., 1968
United Kingdom	1969–1971	201	GLC	0.5	1.8	2.5	72	Abbott et al., 1972
USSR		41	TLC	4.33	3.73			Vas'kovskaja & Komarova, 1967
USSR		197	TLC	5.3–7.57	3.2–7.1			Vas'kovskaja, 1969
Africa								
Kenya						5.4		Wassermann et al., 1972a
Nigeria	1967	43	GLC	5.4	3.1	8.8		Wassermann et al., 1968b
Nigeria	1969	41	GLC	2.1	2.8	6.5	57	Wassermann et al., 1972d
South Africa						5.94	43	Wassermann et al., 1970b
South Africa						7.16		Wassermann et al., 1970b
Uganda						2.9		Wassermann et al., 1974a
Asia								
India (Delhi area, civilian)	1964	67	colour	17	10	26	39	Dale et al., 1965
India (other cities, military)	1964	16	colour	8	5	13	37	Dale et al., 1965
India		94	GLC			13.8[b]	42	Ramachandran et al., 1974
Israel	1963–1964	254	colour	8.5	10.7	19.2	56	Wassermann et al., 1965
Israel	1965–1966	71	colour	4.6				Wassermann et al., 1967
Israel	1965–1966	133	colour	8.2				Wassermann et al., 1967
Israel	1967–1971	63	GLC	3.0	10.6	14.4	74	Wassermann et al., 1974b
Japan	1968–1969	241	GLC	0.6	1.8	2.4	75	Curley et al., 1973
Japan	1969–1970	74				6.92		Nishimoto et al., 1970
Japan	1970					4.499		Suzuki et al., 1973
Japan	1971					2.694		Suzuki et al., 1973
Japan	1971	30				12.895		Kasai et al., 1972
Japan	1972					4.001		Suzuki et al., 1973
Japan	1972	42				5.992		Kasai et al., 1972
Japan	1973					6.44		Kawanishi et al., 1973
Japan	1974					6.87		Inoue et al., 1974
Pakistan		60				25.0		Mughal & Rahman, 1973
Thailand	1969–1970	77				12.6		Wassermann et al., 1972c
Oceania								
Australia	1965	53	GLC	0.77[b]	1.03[b]	1.81[b]	57	Bick, 1967
Australia	1965–1966	46	colour	3.6	6.6	10.2	64	Wassermann et al., 1968a
Australia	1965–1966	12	GLC	3.0	7.1	10.5	68	Wassermann et al., 1968a
Australia	1971	75	GLC			4.94		Brady & Siyali, 1972
New Zealand	1966	52	GLC	1.6	4.2	5.8	72	Brewerton & McGrath, 1967
New Zealand	1965–1969	254	GLC	3.6	11.0	14.6	75	Copplestone et al., 1973

[a] *p,p'*-DDT and *o,p'*-DDT only. Total as DDT includes TDE (DDD) and other forms when given, which are not shown in this table.
[b] Gas–liquid chromatography.
[c] Not detected.
[d] These 28 samples were also tested for DDT and DDE content by a colorimetric method, and the results are included in the 130 samples listed above.
[e] Perirenal fat.
[f] Mesenteric fat.
[g] Panniculus fat.
[h] Geometric mean.
[i] Lipid basis.
[j] Infants and children.

DDT storage in man corresponds with exposure; in general, exposure tends to be greater in warm climates where there is a greater need for insecticides. This climatic dependence may be observed even within a single country (Hayes, 1975). Where measurements have been made during a sufficiently long period, storage has decreased as the use of DDT, especially that leading to residues in food, has decreased.

The method of DDT analysis shifted in about 1962 from the Schecter-Haller colorimetric method to the gas chromatographic method. However, as noted by Hayes (1975), this made little difference to the overall results. Thus the absolute decrease of total DDT storage and the relative increase of DDE storage observed in some countries is real.

Circumstances peculiar to some subpopulations explain their unusual storage of DDT. Thus, persons living in one of the contiguous states of the USA stored significantly less DDT than other persons in the state because they did not eat meat (Hayes et al., 1958). Even less storage of DDT was observed in Eskimos whose diet contained an unusually high proportion of meat obtained from wildlife in which no DDT and almost no DDE could be detected (Durham et al., 1961).

No significant difference was found in the concentration of DDT stored in the fat of different parts of the body (Hayes et al., 1958; Casarett et al., 1968).

DDE in fat. The accumulation of DDE relative to total DDT-related compounds is best illustrated in man. Of the total DDT stored in the fat of workers exposed to technical DDT (about 4% DDE) for 11–19 years, only 38% was in the form of DDE, and, of course, some of that DDE came from their diets which included meat (Laws et al., 1967). In India, where many people avoid meat but may consume milk, cheese, and eggs, 34–41% of total DDT was DDE (Dale et al., 1965). In the USA, during a time when DDT residues in food were decreasing, the proportion of total DDT in the form of DDE increased from about 60% in 1955 to about 80% in 1970; during the same interval the concentration of total DDT in body fat decreased from about 15 mg/kg to slightly less than 10 mg/kg (see Table 9). Thus, a low proportion of DDE indicates a relatively high intake of DDT, a relatively low intake of DDE residues (e.g. in food), and relatively few years for the metabolism of stored DDT to DDE.

DDT in blood. The finding of DDT and related compounds in blood (usually serum) of the general population of different countries is shown in Table 10. Compared to body fat, blood has been analysed for DDT in fewer countries and for fewer years. Some of the same differences between populations and time periods observed in connexion with DDT in fat have been detected in connexion with DDT in blood, but the differences are less marked.

Table 10. Concentration mg/litre of DDT-related compounds in the blood of members of the general population

Country	Year	No. of samples	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p*-TDE(DDD)	*o,p*-TDE(DDD)	Total DDT equivalent	DDE as % total	Reference
North America											
Canada									0.032		Brown & Chow, 1975
USA (Atlanta)	1965	10	0.0119	0.0013	0.0257				0.0418	68.6	Dale et al., 1966b
USA (Atlanta)	1966	10[m]							0.0746		Dale et al., 1967
USA (Atlanta)	1966	10[f]							0.0360		Dale et al., 1967
USA (Louisiana)	1966–1967	53[a]	0.00182	0.00182	0.00265	0.00265	0.00062	0.00062	0.00501	59.0	Selby et al., 1969
USA (4 states)	1967	64	0.00335	0.00044	0.00837	0.00096	0.00032	0.00000	0.01425	78.0	Yobs, 1969 (unpublished data)
USA (3 states)	1968	106	0.00342	0.00006	0.00927	0.00000	0.00004	0.00009	0.01397	73.4	Yobs, 1969 (unpublished data)
USA (Idaho)	1967–1968	1000	0.0047		0.0220		0.0002		0.02940	83.5	Watson et al., 1970
USA (Atlanta)	1969	30[b,c]	0.0046	0.0011	0.0062	0.0003	0.0014		0.0144	50.0	Curley et al., 1969
USA	1968	5[d]							0.0050		Curley & Kimbrough, 1969
USA	1968	10[d]							0.0205		Curley & Kimbrough, 1969
USA (Florida)		45[e]			0.0108						O'Leary et al., 1970b
USA (Florida)		107[g]			0.0152						O'Leary et al., 1970b
USA (Florida)	1970	26							0.03169		Radomski et al., 1971a
South America											
Argentina	1970	20[h]							0.01934		Radomski et al., 1971b
		18[i]							0.01327		Radomski et al., 1971b
		19[j]							0.00869		Radomski et al., 1971b
Brazil	1973?	15[f]	0.0189		0.0237				0.0453		Schvartsman et al., 1974
Brazil	1973?	15[c]	0.0118		0.0104				0.0234		Schvartsman et al., 1974
Brazil	1974?	30	0.145	0.026	0.155				0.336		Almeida et al., 1975
Brazil	1974?	11	0.083		0.117				0.194		Almeida et al., 1975
Brazil	1974?	20	0.086		0.121				0.212		Almeida et al., 1975

Table 10—*continued*

Europe											
Bulgaria	1971–1976	171							0.039[a]		Rizov, 1977
Hungary	1967–1968	120							0.034		Czeglédi-Janko, 1969
Poland									0.172[d]		Jonczyk, 1970
Poland	1972	15							0.030	63	Bojanowska et al., 1973
Switzerland		13							0.0209		Zimmerli & Marek, 1973
Asia											
Israel	1975	29	0.0133	0.0112	0.0195	0.0112	0.0087	0.0072	0.0740	47	Polishuk et al., 1977
Japan	1970	10							0.011		Tokutsu et al., 1970
Japan	1971								0.005		Kojima et al., 1971
Japan	1971	138							0.0183		Kasai et al., 1972
Japan	1971								0.0093		Yamagishi et al., 1972
Japan	1971								0.0285		Kaku, 1973
Japan	1972								0.001–0.078		Study Group, 1972
Japan		37							0.0437[d]		Nawa, 1973
Japan[k]									0.1358		Hara et al., 1973
Japan[c]									0.0210		Hara et al., 1973
									0.0779		Abe et al., 1974
Oceania											
Australia		52							0.0172		Slyali, 1972
Australia		47							0.0169		Ouw & Shandar, 1974

[a] Geometric mean.
[b] Mean of positive values only.
[c] Cord blood from term infants.
[d] Maximal value.
[e] White women.
[f] Female.
[g] Black women.
[h] Adults.
[i] 6–11 years old.
[j] 1–5 years old.
[k] Maternal blood.
[m] Mile.

There is a small but statistically significant decrease in the concentrations of DDT-related compounds in the plasma of women 1–6 days postpartum compared with the same women early in pregnancy. Most of the decrease seems to occur during about the last 10 days before delivery (Curley & Kimbrough, 1969). In a similar way, the concentration of DDT-related compounds in various tissues of women at the time of Caesarean section or normal delivery is less than in nonpregnant women in the same community (Polishuk et al., 1970).

The concentration of *p,p'*-DDE is remarkably constant throughout the day, but minor increases in it and in *p,p'*-DDT may occur after a meal (Radamski et al., 1971a).

In so far as can be judged from the bar graphs presented by Griffith & Blanke (1975), the concentrations of DDT-related materials they found in postmortem blood were similar to the concentrations reported in Table 10 for fresh blood except that postmortem blood contained more TDE (DDD).

DDT and related compounds in other tissues. DDT has been found in some samples of all human organs. Results reported in the USA and USSR are shown in Tables 11 and 12, respectively. Results in Table 11 are based on the wet weight of the tissues. Results in Table 12, presumably, are based on the weight of extractable lipid, but the paper does not specify this; whatever the basis, the paper reports concentrations of DDT and DDE in

Table 11. Average concentrations[b] of organochlorine insecticides in various tissues from autopsies of 44 members of the general population[a]

Tissue	No. analysed	Lipid content (%)	DDT (mg/kg)	DDE (mg/kg)	TDE (DDD) (mg/kg)	Heptachlor epoxide (mg/kg)	Dieldrin (mg/kg)	Total + SE[c] (mg/kg)	
Perirenal fat	30	55.7	1.33	4.64	0.0110	0.0220	0.0300	6.03	±5.30
Mesenteric fat	29	54.2	1.35	4.40	0.0470	0.0320	0.0630	5.89	±4.98
Panniculus fat	30	60.6	1.16	4.48	0.0180	0.0270	0.0270	5.71	±5.25
Bone marrow	19	20.6	0.411	2.08	0.0760	0.0040	0.620	2.63	±2.21
Lymph node[d]	11	8.6	0.892	1.38	0.0100	0.0001	0.0190	2.30	±4.52
Adrenal	18	10.5	0.125	0.875	0.0570	0.0012	0.0060	1.06	±1.31
Kidney	38	3.2	0.0827	0.209	0.0022	0.0009	0.0056	0.300	±0.651
Liver	42	2.1	0.0467	0.200	0.0326	0.0019	0.0037	0.285	±0.369
Brain	32	7.9	0.0105	0.0831	0.0020	0.0002	0.0031	0.989	±0.171
Gonad	36	1.3	0.0150	0.0688	0.0015	0.0001	0.0021	0.0875	±0.103
Lung	25	0.7	0.0147	0.0585	0.0009	0.0003	0.0022	0.0766	±0.125
Spleen	27	0.6	0.0112	0.0305	0.0031	trace	0.0021	0.0469	±0.074

[a] From: Cassarett et al. (1968).
[b] Wet tissue basis.
[c] SE = standard error of the mean.
[d] Tracheobronchial lymph nodes.

Table 12. Average DDT and DDE contents in certain human organs[a]

Organ	Content limits	% of positive observations	DDT (mg/kg)	DDE (mg/kg)
Heart muscle	1.0–12.0	77.0	3.69	4.05
Liver	1.0–20.0	85.5	4.62	4.02
Kidney	1.0–12.0	71.0	2.99	3.44
Spleen	2.0–20.0	57.0	2.86	2.86
Pancreas	2.0–15.0	75.0	3.0	2.62
Adrenal	2.0–10.0	84.3	3.84	3.7
Thyroid	2.0–8.0	59.0	1.85	1.85

[a] From: Vas'kovskaja (1969).

the range of 10.0–35.0 mg/kg in the heart muscle, liver, and kidneys of 2 persons who had had direct contact with pesticides. Although concentrations of chlorinated compounds in liver, kidney, brain, and spleen of persons from Ferrara Province, Italy, were generally higher than those in the USA (Prati et al., 1972), the differences were not great.

DDT and related compounds in the tissues of infants. The transfer of DDT to the fetus was observed by Denés (1962) and confirmed by many others (Curley et al., 1969; Zavon et al., 1969; Komarova, 1970; O'Leary et al., 1970a,b,c). Typical findings are shown in Table 13. It appears that the placenta is partially protective; the concentrations of DDT and DDE are lower in cord blood than in corresponding maternal blood (O'Leary et al., 1970b; Schvartsman et al., 1974). The report by Engst et al. (1970) that infants lose a part of their DDT stores during the first few months of life, as a result of rapid growth, is not necessarily contradictory to the observation that at birth they have less than their mothers, but it must be said that the values reported by Engst et al. (1970) were relatively high for infants (see also section 5.2).

Storage in relation to disease. A review (Hayes, 1975) indicates that there is no agreement in the literature regarding the effect of health on the storage of chlorinated hydrocarbon insecticides. Some investigators have not found any difference in the concentration of DDT in adipose tissue taken by biopsy or during minor elective surgery in contrast to that taken at autopsy. Some investigators, who used only autopsy samples, found no relationship between storage of chlorinated hydrocarbon insecticides and the cause of death. Others, of whom the first was Deichmann (Deichmann & Radomski, 1968; Radomski et al., 1968), have reported that storage was 1.7–7.6 times greater in persons dying of cirrhosis, atherosclerosis, hypertension, idiopathic amyloidosis, and certain forms of cancer. Weight loss was not really ruled out in these studies. Its importance was emphasized by Casarett et al. (1968), who found that disease did not influence concentrations on a wet weight basis but only on a lipid basis; samples with

Table 13. The range and mean of measurable concentrations of various organochlorines (mg/kg) in different tissues from stillborn infants[a]

Tissues received		*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-TDE (DDD)	α-HCH	β HCH	γ-HCH	Heptachlor	Dieldrin
10 adipose	measurable concentration	3	4	8	0	6	3	6	3	4	3
	range	0.16–2.15	0.35–11.47	0.16–3.19	—	0.23–14.17	0.09–0.24	0.14–0.44	0.09–0.14	0.07–0.51	0.09–0.35
	mean	0.88	3.39	1.22	—	3.17	0.14	0.26	0.11	0.32	0.24
	SE ±	0.63	2.70	0.38	—	2.22	0.05	0.05	0.02	0.10	0.08
8 spinal cord	measurable concentration	1	1	2	0	2	0	1	1	0	1
	range	0.47	0.47	0.30–1.16	—	0.31–0.70	—	0.17	0.10	—	0.09
	mean	—	—	0.73	—	0.51	—	—	—	—	—
	SE ±	—	—	0.43	—	0.20	—	—	—	—	—
8 brain	measurable concentration	3	1	4	0	3	3	1	1	1	2
	range	0.28–0.99	0.84	0.25–1.47	—	0.20–1.22	0.04–0.49	3.81	0.06	0.13	0.84–0.86
	mean	0.56	—	0.65	—	0.64	0.19	—	—	—	0.05
	SE ±	0.22	—	0.28	—	0.30	0.15	—	—	—	0.01
9 adrenals	measurable concentration	3	2	6	0	3	2	3	3	2	1
	range	1.28–1.65	0.36–1.05	0.13–1.96	—	0.91–1.45	0.40–0.57	0.12–0.71	0.20–0.53	0.46–1.00	0.92
	mean	1.48	0.71	1.05	—	1.11	0.49	0.37	0.33	0.73	—
	SE ±	0.11	0.35	0.28	—	0.17	0.09	0.18	0.10	0.27	—
10 lungs	measurable concentration	4	0	6	0	5	5	3	3	3	2
	range	0.57–1.01	—	0.25–1.35	—	0.31–1.05	0.07–0.69	0.05–0.18	0.05–0.25	0.08–0.31	0.27–0.72
	mean	0.79	—	0.85	—	0.75	0.25	0.12	0.12	0.17	0.50
	SE ±	0.11	—	0.22	—	0.13	0.11	0.04	0.07	0.07	0.23

Table 13—*continued*

10 heart	measurable concentration	4	2	4	0	4	3	3	3	4	3
	range	1.04–4.17	0.57–0.68	1.27–4.79	—	1.02–5.82	0.23–0.52	0.15–0.31	0.19–0.27	0.30–1.56	0.08–1.02
	mean	2.17	0.63	2.74	—	3.27	0.33	0.22	0.22	0.80	0.49
	SE ±	0.69	0.06	0.74	—	1.10	0.09	0.05	0.03	0.30	0.28
10 liver	measurable concentration	5	3	6	0	6	3	4	4	3	2
	range	0.15–1.59	0.22–3.42	0.22–2.45	—	0.19–2.14	0.21–0.32	0.03–0.20	0.05–0.36	0.03–1.67	0.16–0.22
	mean	0.79	1.32	0.98	—	1.01	0.25	0.11	0.24	0.68	0.19
	SE ±	0.24	1.05	0.34	—	0.29	0.04	0.04	0.07	0.50	0.03
9 kidney	measurable concentration	4	3	6	0	5	4	5	4	3	3
	range	0.62–7.60	0.29–2.07	0.11–9.78	—	0.48–9.12	0.11–1.45	0.06–0.61	0.11–0.69	0.19–1.14	0.06–0.50
	mean	3.71	1.38	3.57	—	3.84	0.82	0.29	0.39	0.70	0.34
	SE ±	1.70	0.55	1.72	—	1.87	0.32	0.12	0.14	0.28	0.14
8 spleen	measurable concentration	3	2	3	1	3	1	1	2	5	3
	range	0.48–1.04	0.45–2.94	0.60–1.05	0.29	0.18–0.91	0.21	0.16	0.17–0.18	0.10–0.52	0.18–0.51
	mean	0.80	1.70	0.86	—	0.56	—	—	0.18	0.35	0.31
	SE ±	0.17	1.25	0.13	—	0.21	—	—	0.005	0.08	0.10
3 pancreas	measurable concentration	1	0	1	1	0	0	0	0	0	0
	range	0.49	—	0.23	0.08	—	—	—	—	—	—
	mean	—	—	—	—	—	—	—	—	—	—
	SE ±	—	—	—	—	—	—	—	—	—	—

[a] From: Curley et al. (1969).

the highest levels of DDT on a lipid basis came from persons who not only had cancer but who were emaciated and had widespread abnormality of the liver. Factors other than emaciation may be important in some conditions. For example, Oloffs et al. (1974) found that the DDT concentration in fat was not influenced by cirrhosis, but that the DDT concentrations in liver were significantly higher in cirrhotic livers with severe fatty infiltration and significantly lower in those with marked fibrosis or bile stasis.

That increased storage of DDT is a result and not a cause of the diseases in which it sometimes occurs is shown by the fact that persons with extensive occupational exposure average 10 times more storage than the highest values reported in connexion with disease. However, they do not exhibit any predisposition to the diseases in question, and these diseases have shown no age-specific increase in incidence related to the introduction and use of insecticides.

6.2.2 Animal studies

A detailed review of the literature (Hayes, 1959) shows that a number of facts about the distribution and storage of DDT were established early either by single, classical papers (now fully confirmed) or by correlation of contributions from several laboratories. The major results may be summarized as follows:

(*a*) DDT is stored in all tissues. Storage of the compound in blood, liver, kidney, heart, and the central nervous system was reported by Smith & Stohlman (1944).

(*b*) Higher concentrations of DDT are usually found in adipose tissue than in other tissues (Ofner & Calvery, 1945).

(*c*) Rats store DDT in their fat at all accurately measurable dietary levels including the unintended residues in standard laboratory feeds.

(*d*) Following repeated doses, storage in the fat increases rapidly at first and then more gradually until a peak or plateau is reached (Laug et al., 1950). It was recognized that repeated doses at a moderate rate could result in greater total storage of DDT in the fat than a single dose at the highest rate that can be tolerated or even a single dose at a rate that frequently is fatal.

(*e*) By plotting animal data published no later than 1950, it is possible to show that, when other factors are kept constant, the equilibrium storage of DDT in each tissue varies directly with the daily dosage.

(*f*) However (with the apparent exception of the dog) storage in the fat, and perhaps in other tissues, is less extensive in relation to dosage at higher dietary levels (Fig. 1).

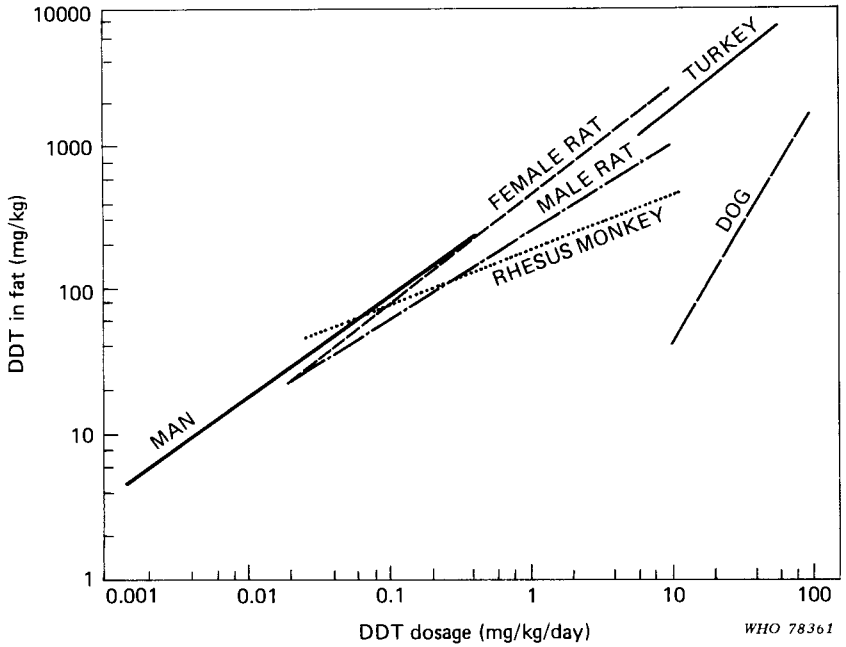


Fig. 1. Storage of DDT in fat of several species as a function of dosage (From: Hayes, 1975).

(*g*) The rat, apparently, tends to lose a part of the DDT it has stored in fat at a peak level, reached in about 6 months, even though the same diet is continued.

(*h*) There is a measurable, although not great, difference between the storage pattern of different species (Fig. 1).

(*i*) At higher dosage levels but not at ordinary residue levels, the female rat consistently stores more DDT in its fat than the male, when offered the same diet (Fig. 1). The difference is only accounted for in part by the greater food intake of the female and must depend to some extent on more rapid biotransformation in the male. Other species show little or no sex difference.

(*j*) The amount of DDT stored in the tissues gradually diminishes if exposure to the compound is discontinued or reduced.

It is interesting to note that, even in the early studies, there was satisfactory agreement between different authors and, in fact, between different laboratories. Later studies have amplified some of the findings.

Adams et al. (1974) observed that about the same concentrations of DDT and related compounds are stored by male rats and by females that reproduce successfully. The lower storage in mated females probably is

accounted for by transfer to the young via the placenta and the milk. However, other factors may be involved; the disposal of the increased DDT taken in by the female rat as a result of her high food intake during lactation has not really been accounted for.

When DDT, some of its analogues, and several other organochlorine insecticides were fed to male and female rats for 4 generations, there was little variation in storage of the materials, from one generation to another, and no evidence of a continuing increase in succeeding generations (Adams et al., 1974).

Distribution to the fetus

In animals, the concentrations of DDT in the blood and other tissues of the fetus are lower than those in the corresponding tissues of the mother (Dedek & Schmidt, 1972). The same is true in man (see section 5.2).

Interaction with other compounds

There was no evidence from studies on rats that dieldrin influenced the storage of DDT and its metabolites, even though DDT caused a marked reduction in storage of dieldrin (Street, 1964). However, Deichmann et al. (1971a) reported that the administration of capsules of aldrin (which is metabolized to dieldrin) to male and female dogs that had reached a steady state of DDT storage caused a rapid and progressive increase in the concentration of DDT, DDE, and TDE (DDD) in their blood and fat. The dosage of DDT was 12 (mg/kg)day and that of aldrin was 0.3 (mg/kg)day. Control dogs that continued to receive DDT but never received aldrin maintained a plateau of DDT storage. It is not clear whether the different results in rats and dogs were due to species, to some unrecognized difference between dieldrin and aldrin, to the fact that the rats received lower dosages, or to some unidentified factor.

Studies of the distribution of DDT in various lipid fractions that are based on tissue extracts obtained with one or more organic solvents, such as those of Kuz'minskaja et al. (1972), are difficult to interpret because there is no way of determining how much of the material was initially associated with protein.

DDE

DDE constitutes about 4% of technical DDT. Most species convert some of the DDT they ingest to DDE. Finally, most species, including man, store DDE more tenaciously than they do DDT, the greater part of which is metabolized by a different pathway to DDA and excreted more rapidly. The result is that DDE, expressed as a percentage of total DDT-related compounds, increases in individuals after DDT intake decreases, and also increases in successive steps of the food chain.

Apparently, the rhesus monkey is an exception. Monkeys store DDE when it is fed to them but, when feeding stops, the rate of loss of DDE stored in fat is more rapid than that of DDT (Durham et al., 1963). Whether it is relative inability to form DDE, unusual ability to excrete it, or a combination of both that accounts for the fact that little or no DDE can be found in monkeys fed DDT is not entirely clear.

6.3 Elimination

6.3.1 Human studies

6.3.1.1 *Studies of volunteers*

DDA is the main urinary metabolite of DDT. In man, it was found first in a volunteer by Neal et al. (1946), who reported that, following ingestion of 770 mg of *p,p'*-DDT, excretion rose sharply to 4.0 mg/day during the second 24-h period, decreased rapidly on the third and fourth days, decreased gradually thereafter, but was still above baseline on the fourteenth day. Judging from a graph, the highest concentration was about 2.6 mg/kg.

Much later studies in volunteers, who received smaller but repeated doses, showed that a steady state of excretion was reached after about 6–8 months. During a 56-week period of continued dosing after equilibrium was fully established, the concentration of DDA associated with technical DDT at the rate of 35 mg/man per day varied from 0.18 to 9.21 mg/kg and averaged 2.98 mg/kg, corresponding values for *p,p'*-DDT were 0.40–6.27 mg/kg with a mean of 1.88 mg/kg. Thus, technical DDT was excreted more effectively and stored in man less than *p,p'*-DDT. During the latter half of the dosing period, it was possible, in the 2 groups receiving recrystallized and technical DDT at the rate of 35 mg/man per day, to account for an average of 13% and 16%, respectively, of the daily dose in terms of urinary DDA. The excretion of DDA was relatively constant in each individual, but marked differences were observed between men receiving the same dose. For example, over the period of 56 weeks, the highest rate measured for one man was 0.16 mg/h while the lowest rate for another in the same group was 0.15 mg/h. Their mean rates during this period were 0.089 and 0.269 mg/h, respectively. The difference was highly significant ($P < 0.001$) (Hayes et al., 1971).

6.3.1.2 *Studies of occupationally exposed workers*

Among workers whose DDT intake was estimated to be about 35 mg/man per day, Ortelee (1958) reported that the concentration of DDA in urine ranged from 0.12 to 7.56 mg/litre and averaged 1.71 mg/litre. Among

workers whose exposure was about half as high, Laws et al. (1967) found concentrations from 0.01 to 2.67 mg/litre with a mean of 0.97 mg/litre.

Continuous sampling of a DDT-formulating plant worker's urine showed that excretion of DDA increased promptly, when exposure began on each of 5 consecutive work days and often continued to increase after exposure, sometimes reaching a peak about midnight before decreasing rapidly. On the sixth day, when there was no occupational exposure to DDT, the excretion of DDA continued until a very low level was reached. The highest concentration of DDA reported in this study was 0.68 mg/litre (Wolfe & Armstrong, 1971).

6.3.1.3 *Studies of the general population*

Urine. Cranmer et al. (1969) developed a method for analysing DDA in urine that, for the first time, made it possible to measure the compound in every sample. The range found for a small sample of the population of Florida, USA, was 0.008–0.019 mg/litre, and the mean (0.014 mg/litre was slightly less than 0.02 mg/litre, the lowest concentration detectable by earlier methods. Results for general population studies are shown in Table 14 together with results for various groups of workers and volunteers.

In the general population the urine contained not only DDA but also neutral compounds; the average concentrations reported by Cueto & Biros

Table 14. Urinary excretion of DDA by people in the USA with various degrees of exposure to DDT[a]

Exposure	Year	No. of samples	DDA excretion (mg/kg)		Reference
			Range	Mean	
General population	1954	8	<0.05	—	Hayes et al., 1956
General population	1957	8	<0.02–0.07	—	Hayes et al., 1971
General population	1962	23	<0.02–0.18	—	Durham et al., 1965b
General population	1968	11	0.008–0.019	0.014	Cranmer et al., 1969
Environmental[b]	1962	13	<0.02–0.11	—	Durham et al., 1965a
Subjects applying DDT	1962	11	<0.02–0.17	—	Durham et al., 1965a
Formulators	1957	40	0.12–7.56	1.71	Ortelee, 1958
Makers and formulators	1966	35	<0.01–2.67	0.97	Laws et al., 1967
Volunteers given 3.5 mg/day orally	1953–1954	2	0.10–0.42[b]	0.21[c]	Hayes et al., 1956
Volunteers given 3.5 mg/day orally	1957–1958	6	0.06–1.98[a]	0.23[b]	Hayes et al., 1971
Volunteers given 35 mg/day orally	1953–1954	6	0.69–9.67[b]	2.46[c]	Hayes et al., 1956
Volunteers given 35 mg/day orally	1957–1958	6	0.18–9.21[c]	3.09[d]	Hayes et al., 1971

[a] Slightly modified from Hayes (1975).
[b] Residents living within 500 feet of agricultural application.
[c] Based on all samples after thirty-fifth week of dosage.
[d] Based on all samples from the thirty-fifth to the ninety-third week after dosage started.

(1967) were: *p,p'*-DDT, 0.0007 mg/litre and *p,p'*-DDE, 0.0156 mg/litre. Men with full-time occupational exposure to DDT excreted much more DDA but showed only a statistically insignificant increase in the excretion of DDT and DDE.

These values for the excretion of DDA, DDT, and DDE by different, small groups of people showed an average concentration of 0.0358 mg/litre of DDT-related material. Although the DDT intakes of these particular groups were not measured, the urinary excretion is of such an order of magnitude that it may account for the excretion of all the absorbed DDT.

Milk. As far as the mother is concerned, the secretion of a compound in milk is a form of excretion. For the infant, the milk is an important, if not the sole source of intake of the compound in question. The concentrations of DDT and DDE in milk reported from different countries are listed in Table 15. Especially high values have been reported from Guatemala for 1970 (see Table 16) and from the USSR (Damaskin, 1965). However, additional and more numerous samples taken only four years later in the same and other communities in Guatemala revealed entirely different results. The highest single value observed for total DDT in 1974 was 5.69 mg/litre. The range of average values for different locations was 0.04–0.86 mg/litre. The means for total DDT for the three communities studied earlier were: La Bomba, 0.59 mg/litre; El Rosario, 0.28 mg/litre; and Cerro Colorado, 0.47 mg/litre (Winter et al., 1976). The authors recognized the importance of the agricultural uses of DDT as a potential source of the compound in human milk. However, they attributed the change between 1970 and 1974, almost exclusively, to the substitution of propoxur for DDT in residential spraying to combat malaria.

The medical importance of DDT in human milk depends entirely on the dosage of the compound received by babies. The highest concentration of *p,p'*-DDT ever reported in a single sample of milk and the highest average value from one community (see Table 16) would determine maximum and average intakes of 1.06 and 0.32 (mg/kg) day for newborn babies, assuming an intake of 0.6 litre per day and a weight of 3.36 kg. This average intake is of the same order of magnitude as that encountered by workers who make and formulate DDT. The corresponding maximum and average intakes of total DDT-related material for infants in Guatemala would be 2.18 and 0.73 (mg/kg) day, values not strictly comparable to those of the workers because the intake of the babies includes so much more DDE, a less toxic compound. Neither of the papers cited concerning DDT in human milk in Guatemala mentioned any indication of injury to babies. Absence of injury to babies would be predicted from studies of the most heavily exposed workers and also from studies regarding the effect of age on the susceptibility of animals to DDT (see section 7.3.2).

Table 15. Concentration of DDT-derived material in human milk

Country	Year	No. of samples	Method of analysis	DDT (mg/litre)	DDE as DDT (mg/litre)	Total as (mg/litre)	DDE as DDT (% of total)	Reference
North America								
Canada	1967–1968	147	GLC	0.032	0.097	0.139	—	Ritcey et al., 1972
Canada		15	GLC	0.006–0.032	—	0.019–0.035	—	Musial et al., 1974
USA	1950	32	colour		—	0.13	—	Laug et al., 1951
USA	1960–1961	10	colour	0.08	0.04	0.12	33	Quinby et al., 1965b
USA	1962	6	colour	0–0.12[a]	0.025[a]	0.37[b]	—	West, 1964
USA	1968	?	GLC	0.026	0.047	0.078	60	Curley & Kimbrough, 1969
USA	1970	53	GLC	0.022	0.083	0.101	80	Kroger, 1972
USA	1970–1971	101	GLC	—	—	0.17		Wilson et al., 1973
USA	1971–1972	40	GLC			0.126		Savage et al., 1973
USA	1973–1974	57	GLC	0.092	0.260	0.344	76	Strassmann & Kutz, 1977
USA	1974	38	GLC			0.447		Woodard et al., 1976
USA	1974	14	GLC			0.075		Woodard et al., 1976
USA	1975	7	GLC			0.323		Woodard et al., 1976
Europe								
Belgium	1968	20	GLC	0.05	—	—	—	Heyndrickx & Maes, 1969
Czechoslovakia	1968	—	—	0.101	—	—	—	Hruska, 1969
Czechoslovakia		393	TLC	0.097	0.112	0.209	54	Suvak, 1970
England	1963–1964	19	GLC	0.05	0.08	0.13	62	Egan et al., 1965
France	1971–1972					3.24[c]		Luquet et al., 1974
France	1972?					3.24[c]		Luquet et al., 1974
German Democratic Republic	1970?					0.569		Adamovic et al., 1971
German Democratic Republic	1969	57				0.23		Engst & Knoll, 1972
German Democratic Republic	1970	18				0.16		Engst & Knoll, 1972
German Democratic Republic	1971	96				0.32		Knoll & Jayarman, 1973a 1973b
Germany, Federal Republic of	1970?	43	GLC	0.031	0.090	0.121	74	Acker & Schulte, 1970

Table 15—*continued*

Germany, Federal Republic of	1971?					0.121		Pfeilsticker, 1973
Hungary	1963	10	colour	0.13–0.26[a]	—	—	—	Denés, 1964
Italy	1965?	2	GLC	0.001	0.055	0.056		Kanitz & Castello, 1966
Netherlands	1969	50	GLC	0.9[c]	1.8[c]	2.7[c]	66	Tuinstra, 1971
Poland	1966	26	colour	0.27	—	—	62	Bronisz & Ochynski, 1968
Poland	1967	25	colour	0.40	—	—	58	Bronisz & Ochynski, 1968
Poland	1970?	40	GLC	0.08	0.19	0.28	71	Kontek et al., 1971
Portugal	1972	168	GLC			0.326		Graca et al., 1975
Romania	1968?	100	colour	0.054–0.749	0.026–8.30	0.080–9.05	—	Unterman & Sirghie, 1969
Sweden	1967?	—	—	—	—	0.117	—	Lotroth, 1968
Sweden	1967–1969	22	GLC	0.039	0.076	0.115	63	Westoo et al., 1970
USSR	1964	16	colour	1.22–4.88	—	—	—	Damaskin, 1968
USSR	1968?	4505	—	0.1–1.0	—	—	—	Gracheva, 1969
USSR	1969?	—	—	0.09	—	0.14	—	Gracheva, 1970
USSR	1967?	370	GLC	0.1	—	—	—	Komarova, 1970
Asia								
Israel	1975	29	GLC	0.02	0.03	0.07	44	Polishuk et al., 1977
Japan	1970?	10				0.071		Tokutsu et al., 1970
Japan	1970?	5				0.160		Takeshita & Inuyama, 1970
Japan	1970?	10				0.120		Takeshita & Inuyama, 1970
Japan	1971?					0.04		Kojima et al., 1971
Japan	1971?					0.04		Kojima et al., 1971
Japan	1971?	59				0.019–0.105		Kato et al., 1971
Japan	1971?	14				0.047		Sugaya et al., 1971
Japan	1971	43	GLC	0.095	0.084	0.179	47	Hidaka et al., 1972
Japan	1971	454	GLC			0.0607		Hayashi, 1972a, 1972b
Japan	1971–1972	398				0.0626		Hayashi, 1972a, 1972b
Japan	1971–1972	398		0.0562	—	—	—	Anonymous, 1972
Japan	1971					0.044		Yamagishi et al., 1972
Japan		30				2.0[a]		Mizoguchi et al., 1972
Japan		54				0.035		Taira et al., 1972
Japan	1971–1972	5				0.027		Nagai, 1972
Japan	1971–1972	5				0.037		Nagai, 1972
Japan	1971–1972	5				0.016		Nagai, 1972
Japan	1971–1972	5				0.037		Nagai, 1972
Japan		30				0.033		Oura et al., 1972

Table 15—*continued*

Country	Year	No. of samples	Method of analysis	DDT (mg/litre)	DDE as DDT (mg/litre)	Total as (mg/litre)	DDE as DDT (% of total)	Reference
Japan	1971–1972	123				0.105		Kawai et al., 1973
Japan	1971–1972					0.038–0.075		Kamata, 1973
Japan	1970					3.780[c]		Suzuki et al., 1973
Japan	1971					3.592[c]		Suzuki et al., 1973
Japan	1972					3.822[c]		Suzuki et al., 1973
Japan	1973					0.0854		Kamata, 1974
Oceania								
Australia	1970	1	GLC	—	—	0.014[d] 0.007[e] 0.066[f]		Newton & Greene, 1972
		67	GLC	0.036	0.105	0.141		
Australia								
(Brisbane)	1971–1972	20	GLC			0.288		Miller & Fox, 1973
(Mareeba)	1971–1972	20	GLC			0.415		
Australia		45	GLC			0.064		Siyali, 1973
Australia		22	GLC	0.010	0.068	0.078		Stacy & Thomas, 1975
Papua New Guinea	1972	16	GLC					
(Kar Kar Island)				0.002	0.002	0.004	50	Hornabrook et al., 1972
Papua New Guinea	1972	19	GLC					
(Sepik district)				0.008	0.007	0.015	47	Hornabrook et al., 1972

[a] Range of values for milk containing 4% fat containing 3.3–6.6 ppm.
[b] Maximal value.
[c] Concentration in milkfat.
[d] At beginning of feeding, 1.8% fat.
[e] At middle of feeding, 1.2% fat.
[f] At end of feeding, 5.1% fat.

Table 16. Ranges, means, and standard errors of the concentrations of organochlorine insecticides in the milk of women in three towns in Guatemala[a]

Compound	La Bomba 1970 $n = 10$	El Rosario 1970 $n = 27$	Cerro Colorado 1971 $n = 9$
p,p'-DDT (mg/litre)	0.23–4.95 (1.00 ± 0.38)	0.16–2.24 (0.77 ± 0.10)	0.49–5.94 (1.78 ± 0.56)
p,p'-DDE (mg/litre)	0.12–6.36 (1.02 ± 0.58)	0.28–3.10 (0.99 ± 0.14)	0.60–6.13 (2.10 ± 0.61)
p,p'-TDE(DDD) (mg/litre)	tr[b]–0.16 (0.03 ± 0.02)	0.01–0.09 (0.02 ± 0.004)	0.05–0.11 (0.07 ± 0.01)
o,p'-DDT (mg/litre)	tr–0.29 (0.09 ± 0.03)	0.01–0.18 (0.06 ± 0.01)	0.06–0.22 (0.12 ± 0.02)
total as DDT (mg/litre)	0.41–11.50 (2.15 ± 1.05)	0.34–4.97 (1.84 ± 0.24)	1.57–12.21 (4.07 ± 1.11)
total HCH (mg/litre)	0.01–0.10 (0.03 ± 0.01)	tr–0.07 (0.007 ± 0.003)	0–0.06 (0.02 ± 0.01)
heptachlor epoxide (mg/litre)	0–0.02 (0.003 ± 0.002)	tr–0.01 (0.007 ± 0.0004)	tr
dieldrin (mg/litre)	tr	tr–0.01 (0.002 ± 0.0005)	

[a] From: Olszyna-Marzys et al. (1973).
[b] tr = trace.

The slightly greater secretion of DDT and much greater secretion of various isomers of HCH by urban mothers (compared to rural mothers) in Japan was attributed to their greater intake of cow's milk (Takano, 1972).

Other routes of excretion. DDT, DDE, and dieldrin are excreted in the bile; the concentrations for five men without special exposure varied as follows: *p,p'*- and *o,p'*-DDT combined, 0.0000–0.0009 mg/litre; *p,p'*-DDE, 0.0005–0.0056 mg/litre; and dieldrin, 0.0000–0.0005 mg/litre. Higher levels were found in the bile of one pest control operator (Paschal et al., 1974).

6.3.2 Animal studies

When large doses of DDT are ingested, some of the compound is not absorbed and is passed in an unaltered state in the faeces. Only traces of unaltered DDT may be found in the faeces when exposure is by any route other than oral. However, true faecal excretion of DDT metabolites was established very early (Wasicky & Unti, 1945; Judah, 1949). In the rat, faecal excretion of DDT exceeded urinary excretion, irrespective of the route of administration (Hayes, 1965). In man, the ratio is obviously different. Although the excretion of DDT-related material in the faeces of man receiving 35 mg/man per day has been reported using colorimetry (Hayes et al., 1956), this result has never been confirmed by gas chromatography, even in connexion with workers whose exposure was heavy and

prolonged. Either DDT metabolites are not excreted by man in the faeces to any great degree, or they are excreted in one or more forms that differ from those already demonstrated in rats.

The bile appears to be the principal source of DDT metabolites in the faeces of rats. When the bile duct was cannulated before intravenous injection of radioactive DDT, 65% of the dose was recovered in the bile, 2% in the urine, and only 0.3% in the faeces (Jensen et al., 1957), and the possibility of some contamination of the faeces by urine could not be excluded.

The different routes of excretion are not unrelated. Burns et al. (1957) found that there was an increase in urinary excretion of radioactive material following ligation of the bile duct in rats fed radioactive DDT. This is an indirect confirmation of the finding by Jensen and his colleagues that most of the metabolites in bile consist of DDA or compounds closely related to it. Although an enterohepatic circulation of the metabolites of DDT has not been directly proved, it seems likely that such a circulation exists, as has been demonstrated for 1,1'-(2,2-dichloroethylidene)-bis[4-ethylbenzene] (Perthane).

Demonstration of the excretion of DDT in milk was first reported by Woodard et al. (1945) in connexion with a dog fed the compound at the rate of 80 (mg/kg) day. Within a short time, excretion of DDT in milk was reported in rats, goats, and cows, and soon afterwards, in women (Laug et al., 1951). Telford & Guthrie (1945) showed that rats fed a diet containing DDT at 1000 mg/kg produced milk that was toxic to their young.

Since the early laboratory studies, the presence of DDT has been demonstrated repeatedly in the milk of cows. A review (Hayes, 1959) showed that cows fed substantial, but nontoxic, residues of DDT commonly excrete 10% or slightly more of the total dose in their milk, and amounts slightly over 30% have been observed.

Further information on the excretion of DDT in human milk is given in section 6.3.1.3. It is of interest to repeat here, however, that lactating women in the general population are apparently in negative DDT balance. That is, they excrete more DDT in their milk each day than they acquire in their food. The difference is small and would not be expected to have much effect on their total body burden of DDT.

Wilson et al. (1946a) showed that DDT was secreted from the skin of a cow maintained on an oral dosage of about 53 (mg/kg) day.

DDA. Because DDA is the main form in which DDT is excreted, it might be expected that, following its direct administration, DDA would be excreted relatively efficiently, and this is true. It was found very early that, during the first few days after oral dosing, rabbits excreted DDA in the urine approximately 15 times faster than animals given DDT at an equivalent dosage. Although the rate of DDA excretion increased somewhat, the rate

of excretion associated with DDT increased more rapidly so that the values differed by a factor of only five after the twentieth day of feeding (Smith et al., 1946).

6.4 Biotransformation

The chemical nature of the chief metabolite excreted in the urine was first elucidated by White & Sweeney (1945). Rabbits were given DDT (melting point 107–108° C) at a rate of 100 (mg/kg) day for 6 days per week, and their urine was collected. It contained a considerable amount of organic chloride, whereas normal rabbit urine did not. Using the organic chloride test to evaluate different methods of extraction, the authors were able to isolate a crystalline material containing 25.37% chlorine and melting at 166–166.5° C. The crystals were shown to be DDA (see Table 1). The product obtained from the urine was identical to that synthesized from glyoxylic acid and chlorobenzene and with a compound obtained through the chemical degradation of DDT. Identity of the 3 compounds and, therefore, their true chemical nature, was established by the determination of melting points, mixed melting points, elementary analysis, and X-ray powder diffraction patterns, as well as by demonstrating the similarity of the decarboxylation products of the three original materials. Only 80–85% of the total organic chloride of the rabbit urine was found to be soluble in alkali and in bicarbonate. For this and other reasons it was considered possible that DDA was not the only chlorinated organic compound present.

Later work by many authors has confirmed that DDA is the major urinary metabolite of DDT in all mammals including man. It may be added that, in spite of great strides in analytical chemistry, the nature of other urinary metabolites has not been elucidated fully.

The fact that DDE is stored in tissue was first demonstrated by Pearce et al. (1952) in connexion with human fat. The authors pointed out that they did not know whether the compound resulted from partial degradation of DDT residues on plants or whether the DDE was formed during the process of digestion or after absorption. It is now known that some food contains DDE but that man is capable of forming the product from DDT. The exact mechanism of the biotransformation of DDT to DDE remains in doubt.

Pearce et al. (1952) established the identity of DDE by comparing the colorimetric and column chromatographic behaviour of the residue with those of a chemical standard. A second paper from the same laboratory (Mattson et al., 1953) added further details confirming the identity of the compound. Later investigations have confirmed the identity of DDE by infrared spectrometry and by gas chromatography.

That portion of the metabolism of DDT that leads to DDA has been clearly elucidated by Peterson & Robinson (1964), who gave evidence for the sequence of changes shown in Fig. 2. Organ perfusion studies have indicated that the liver is capable of the biotransformation of DDT, DDE, TDE, DDMU, and DDMS, while the kidney transforms DDMS, DDNU, and DDOH (Datta & Nelson, 1970). Cultures of embryonic lung cells are capable of metabolizing DDT to DDA via DDD (North & Menzer, 1973).

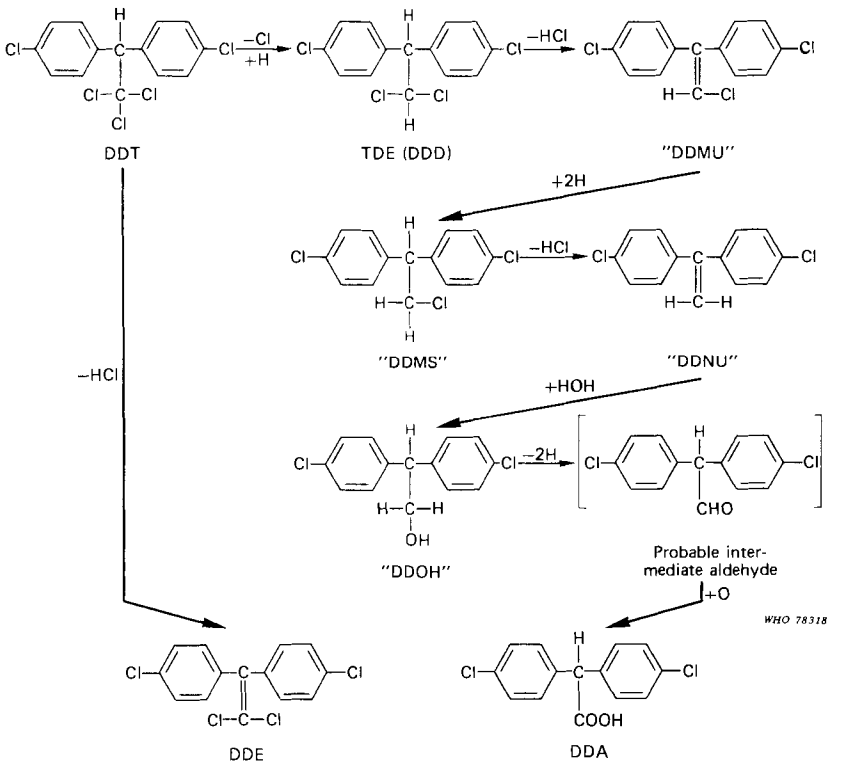


Fig. 2. Biotransformation of DDT (From: Peterson & Robinson, 1964).

When DDA was discovered, it was postulated, on chemical grounds, that DDE was a step in its formation (White & Sweeney, 1945); however, rats that produced both DDE and DDA from DDT were incapable, according to Peterson & Robinson (1964), of forming DDA when fed preformed DDE. This finding was contradicted by Datta (1970) and by Datta & Nelson (1970) who claimed that ^{14}C-*p,p'*-DDE was converted by rats to *p,p'*-DDMU, which then underwent further metabolism to *p,p'*-DDA via the

route shown in Fig. 2. Datta suggested that the predominance of detoxication via DDE or TDE (DDD) might depend on physiological response or the amount of toxicant used. Whatever the reason, the fact remains that DDE is stored more tenaciously than DDT.

Rhesus monkeys fed either technical or *p,p'*-DDT store little or no DDE, although they are fully capable of storing DDE when it is fed preformed (Durham et al., 1963).

The way in which DDE was lost from storage was not clearly understood for a long time. In man (Cueto & Biros, 1967), seal, and guillemot (Jansson et al., 1975) part of it is excreted unchanged, but the fact that its elimination is promoted by the induction of microsomal enzymes (see section 7.1.4.2) strongly suggests that it undergoes metabolism, conjugation, or both. That metabolism does occur was first demonstrated by identification of 2 hydroxylated derivatives of DDE in the faeces of wild seals and guillemots and in the bile of seals (Jansson et al., 1975). When *p,p'*-DDE was fed to rats, the same metabolites and one other were isolated from the faeces, and, within the first 6 days, the metabolites accounted for about 5% of the dose (Sundström et al., 1975). Later a fourth hydroxylated derivative was identified from the faeces of rats fed *p,p'*-DDE. The compounds are *m*-hydroxy-*p,p'*-DDE [1,1-dichloro-2-(*p*-chloro-*m*-hydroxyphenyl)-2-(*p*-chlorophenyl)-ethylene, the major metabolite], *o*-hydroxy-*p,p'*-DDE, *p*-hydroxy-*m,p'*-DDE (the product of an NIH shift), and *p*-hydroxy-*p'*-DDE. A scheme (Fig. 3) involving *m,p*-epoxy-*p,p'*-DDE and *o,m*-epoxy-*p,p'*-DDE was proposed for the formation of these metabolites as well as a fifth metabolite (Sundström, 1977). Neither the fifth metabolite nor the hypothetical intermediate have been isolated.

DDE is metabolized not only to easily excretable phenols but also to *m*-methylsulfone-*p,p'*-DDE. In the blubber of seals from the Baltic, this compound was found in a concentration of 4 mg/kg along with DDE (138 mg/kg), TDE (DDD) (10 mg/kg), DDT (78 mg/kg) and various PCB's and their metabolites (150 mg/kg) (Jensen & Jansson, 1976).

The conversion of *o,p'*-DDT to *p,p'*-DDT has been reported (Klein et al., 1965; French & Jefferies, 1969), but, when the possibility was reinvestigated using ^{14}C-*o,p'*-DDT, no conversion could be detected (Cranmer, 1972). The chromatographic peak closely resembling that of *p,p'*-DDT observed in the earlier studies undoubtedly was due to the presence of a metabolite of *o,p'*-DDT, which may explain the more rapid metabolism of the *o,p'*-isomers that has been observed in rat, man, and perhaps other species. The more rapid excretion of *o,p'*-DDT is explained, at least in part, by the observed ring-hydroxylation of the parent compound in rats (Feil et al., 1973) and chickens (Feil et al., 1975) and of preformed *o,p'*-TDE (DDD) in rats (Reif & Sinsheimer, 1975) and in man (Reif et al., 1974). At least 13 metabolites

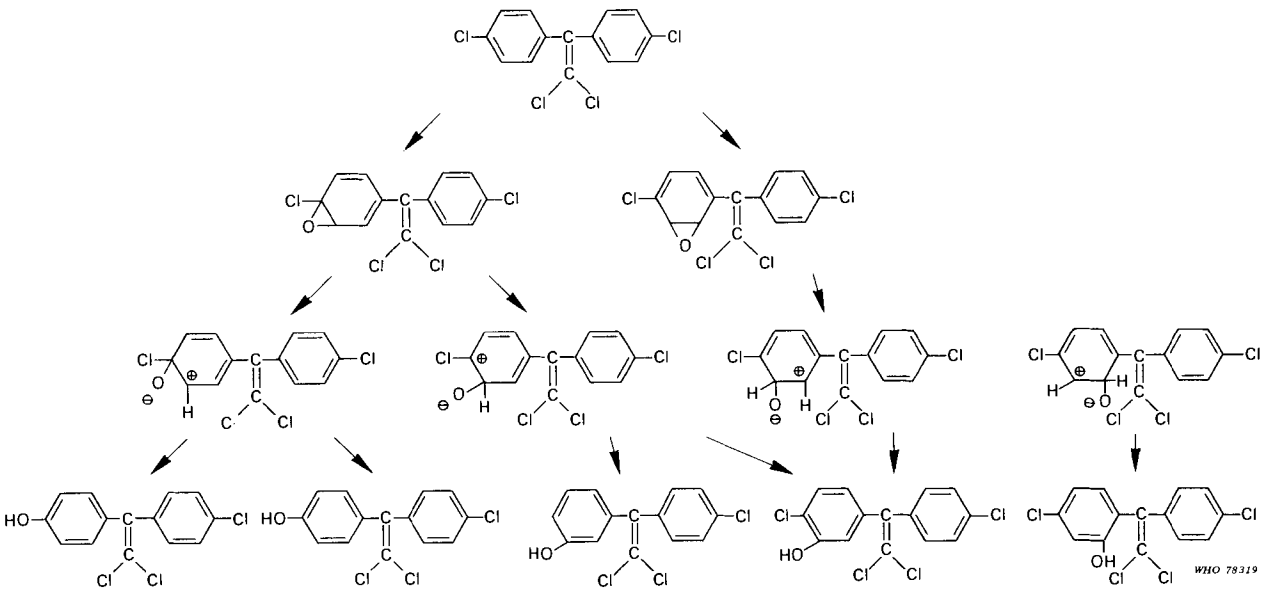


Fig. 3. Biotransformation of DDE (From: Sundström, 1977).

were detected in rats and 15 in chickens. Ring-hydroxylation, which has not been observed with *p,p'*-DDT or *p,p'*-TDE, was present in all species. There were, however, some species differences. For example, *o,p'*-DDE and three hydroxylated *o,p'*-DDE's were found in the excreta of chickens but not in the excreta of rats. In 2 patients with adrenal carcinoma for which they were receiving *o,p'*-TDE at a rate of 2000 mg/day, as much as 46–56% of the daily intake was recovered in the urine following acid hydrolysis. Just over half of the recovered material was in the form of *o,p'*-DDA, but the remainder was in the form of hydroxylated derivatives, specifically *m*-hydroxy, *p*-hydroxy-, *m*-hydroxy-*p*-methoxy-, and *p*-hydroxy-*m*-methoxy-*o,p'*-DDA. Some other hydroxylated compounds were found in trace amounts. All hydroxylation had occurred on the ring that had its chlorine in the ortho position (Reif et al., 1974). When the metabolism of a single 100 mg oral dose of ^{14}C-*o,p'*-TDE was studied in rats, averages of 7.1 and 87.8% of the activity were recovered in the urine and faeces, respectively, within 8 days (Reif & Sinsheimer, 1975). The high recovery indicated rapid excretion with little storage.

The compound identified by Peterson & Robinson (1964) as a "probable intermediate aldehyde" was later synthesized and shown to be highly labile (McKinney et al., 1969), confirming the guess by Peterson & Robinson that it was unlikely to accumulate in tissues in measurable amounts.

Of the compounds shown in Fig. 2 and 3, only DDT, TDE (DDD), DDE, and DDA are commonly reported in the tissues or excreta of animals, including man. The symptomatology produced when the metabolites are administered directly is discussed in section 7.1.1, while uptake, distribution, and elimination of the compounds are discussed in sections 6.1.2, 6.2.2, and 6.3.2, respectively.

Although microorganisms, plants, insects, and birds produce many of the same metabolites that are found in mammals, there are interesting differences. Nearly 20 derivatives (including mammalian metabolites) have been identified, and the chemical structure of several more is still unknown. Some aspects of nonmammalian, as well as mammalian metabolism have been reviewed (Menzie, 1969; Klein & Korte, 1970; Fishbein, 1974; Schroeder & Dorozalska, 1975). The metabolism of microorganisms and plants, as well as that of domestic animals, may influence the composition of DDT-derived residues in human food, but there is no evidence that these residues contain a significant amount of any compound not formed from DDT by human metabolism.

7. EXPERIMENTAL STUDIES ON THE EFFECTS OF DDT

7.1 Animal Studies

7.1.1. Haemopoietic system and immunology

Many early reports reviewed by Hayes (1959) indicated that large doses of DDT might not have any effect on the blood or that they might produce a moderate leukocytosis and a decrease in haemoglobin, with or without a decrease in the concentration of red cells. The leukocytosis probably is secondary to stimulation of the sympathetic nervous system, while the loss of haemoglobin may be nutritional in origin. Later studies have confirmed the early results. A range of haematological variables remained unchanged in squirrel monkeys dosed orally at rates of 0, 0.05, 0.5, 5, and 50 (mg/kg)/day, even though the highest dosage was fatal within 14 weeks (Cranmer et al., 1972).

Immunology. Inasmuch as some compounds are antibiotic, it is logical that some may be probiotic, that is, that they either reduce resistance to infection or increase the virulence of an infecting organism (Hayes, 1975). Far fewer studies have been made of probiosis than of antibiosis. A number of papers have reported one or other possibly probiotic property of DDT, but some of the reports could not be confirmed, and others have not been retested.

It has been claimed that a change in the phagocytic activity of white blood cells is an indication of early intoxication by DDT (Kun'ev, 1965). However, Kaliser (1968) did not find any statistical difference in *in vitro* or *in vivo* phagocytosis of control rats and those receiving DDT by stomach tube at a rate of 0.25 (mg/kg)/day for 31 days. The highest rate at which men who make and formulate DDT in the USA now absorb the compound is about 0.25 (mg/kg)/day.

Rats receiving an aqueous suspension of *p,p'*-DDT of unstated stability at a concentration of 200 mg/litre as their only source of water for over 30 days were reported to develop a lower titre of antibodies to ovalbumen (Wassermann et al., 1969). Rabbits responded to the same treatment with a statistically significant reduction of antibody titre against *Salmonella* and a reduction in antibody titre against sheep red blood cells that was not statistically significant (Wassermann et al., 1971). Both the rats and rabbits showed a decrease in at least one globulin fraction of the blood.

Other reports of changes in immunohaematological indices are those of Semenčeva (1968) and Fridman (1970).

One group of investigators has shown clearly that what at first appeared

to be an immunological response really involved a quite different, predictable effect. Briefly, it was shown that guineapigs sensitized to diphtheria toxoid were less susceptible to anaphylaxis in response to a challenge dose of the toxoid if they were pretreated with DDT at a dosage of only 1–20 mg/kg. Direct measurement of antitoxin production indicated little or no difference between protected and unprotected animals. Furthermore, some protection was given by DDT administered for only 3 days prior to the induction of anaphylaxis (Gabliks et al., 1973, 1975). Further studies showed that DDT treatment reduced the histamine levels in the lungs of both immunized and nonimmunized animals. The number of detectable mast cells was also reduced; this was true whether the count was made in tissues from guineapigs dosed systemically with DDT or in the lungs and mesenteric tissue taken from untreated animals and exposed to DDT *in vitro* at concentrations ranging from 10 to 45 mg/litre. These results indicate that the protection offered by DDT was the result of a reduction of the amount of histamine available for sudden release in response to a challenge dose of toxoid (Askari & Gabliks, 1973). Regardless of exposure to DDT, immunization leads to an increase in detectable mast cells (Gabliks et al., 1975).

7.1.2 Nervous system and behaviour

DDT intoxication in animals was well described by Domenjoz (1944). The first perceptible effect is abnormal susceptibility to fear, with violent reaction to normally subthreshold stimuli. There is definite motor unrest and increased frequency of spontaneous movements. As poisoning increases, hyperirritability, like that seen in strychnine poisoning develops, but convulsions do not appear at this time. A fine tremor, recognizable at first only as a terror reaction, is later present as an intention tremor in connexion with voluntary movement. Then it is present intermittently without observable cause, and finally it is present as a coarse tremor without interruption for as long as several days. Spontaneous movement is limited, and food intake stops so that surviving animals lose weight. In the later stages, especially in some species, there are attacks of epileptiform, tonic-clonic convulsions with opisthotonos.

All the signs are strengthened by external stimuli and become manifest at first through external stimuli. At all stages, the animals show normal position and labyrinth reflexes. The picture of poisoning in mammals recalls the disturbances of movement and tone that are known in human pathology as the amyostatic syndrome.

Symptoms appear several hours after oral administration of the compound, and death follows after 24–72 h. The latent period after intravenous

administration at about the LD_{50} level is approximately 5 min; signs of poisoning reach a maximum level in about 30 min, and survivors are symptom-free in 18 to 24 h. Animals that survive recover completely.

In addition to the features of poisoning already mentioned, Cameron & Burgess (1945) noticed that as rats, guineapigs, and rabbits become sick, they become cold to the touch and show ruffled fur. Some show diarrhoea. These authors found that muscular tremors were preceded by muscular weakness that first occurred in the back and later in the hind legs. The front legs were relatively spared so that animals showing marked weakness of the hind quarters could still drag themselves about. However, several authors have found that the tremor characteristic of DDT poisoning generally starts in the muscles of the face, including the eyelids, and spreads caudally with variable severity until all the muscles are affected. Furthermore, although weakness of hind quarters has been seen by others, it is not a common finding.

Like tremor, coldness of the skin and ruffling of the fur probably represent an indication of disturbed thermal regulation. Apparently it was not until the work of Hrdina et al. (1975) that an increase of almost 3° C in body temperature was reported in rats following a fatal (600 mg/kg) oral dosage of DDT.

Although there is a general similarity in the clinical effects of DDT in all vertebrate species, some characteristic differences exist. Cats show greater extensor rigidity and opisthotonos than other laboratory animals. The stiffness appears first in the distal part of the extremities and later extends to the proximal part and to the trunk. Poisoned cats show marked pilomotor activity. Convulsions in them may become almost continuous. Convulsions are also prominent in dogs as is ataxia. Tremors are so pronounced in rats that it may be difficult to detect clonic convulsions in them. Rats poisoned by DDT show a reddish colour about the eyes just as they do when ill from many other causes. The colour has been attributed to excessive secretion of a porphyrin by the Harderian glands.

Poisoning produced by repeated doses of DDT differs from that produced by a single dose only in so far as the animal may be gradually debilitated, especially by malnutrition. If food intake is maintained, tremor may last for weeks, or even intermittently, for months. If the animals survive a short time after dosing stops, recovery is complete. However, food intake may be interfered with in at least two ways. Tremor and more severe signs may interfere mechanically with eating. Animals offered food containing high concentrations of DDT often eat little or nothing and lose weight rapidly. However, the same animals will show excellent appetites when offered the same kind of food without DDT just after refusing the major portion of the daily ration of contaminated food. Unlike dieldrin and some

other compounds, DDT seems to have little effect on appetite as mediated by the central nervous system; it has a great deal to do with taste.

Animals that have suffered severe weight loss as a result of DDT poisoning may die partly as a result of general debility. In some colonies, at least, they have become prey to secondary infection.

In summary, it may be said that animals that die as the result of repeated large doses of DDT and small animals that die as a complication of starvation following many somewhat smaller doses of DDT show the same signs as those seen in animals killed by one or a few large doses. Even though severely ill, animals that survive a few days after the last of many doses of DDT recover.

Of samples that may be collected from a living animal, the concentration of DDT in serum most accurately reflects its concentration in the brain, the critical tissue. In the rat, levels of 25 mg/kg (wet weight) in the brain are not usually fatal: higher levels tend to be fatal regardless of whether absorption followed one or many doses (Dale et al., 1963; Hayes & Dale, 1964). As reviewed by Hayes (1975), the danger level is approximately the same in several species of birds.

Behavioural changes may be demonstrated in animals receiving DDT, daily, at rates too low to produce illness. Khairy (1959) was able to detect ataxia in the form of changes in gait in rats that had been fed DDT at dietary levels of 100 mg/kg or more for 21 or more days. Gait was recorded by smearing the hind paws of the animals with vaseline, which then recorded their tracks on paper. Gait was recorded in terms of the tangent, that is the ratio of the width and length of step. At a body weight dosage of about 5 (mg/kg)/day the ratio was less than normal, a change the author attributed to an exaggeration of the stretch reflex. At dosages of about 10, 20, and 30 (mg/kg)/day, the ratio was progressivley greater than the normal as a result of broadening of the gait and shortening of the steps. These same dosage levels did not affect problem-solving behaviour or speed of locomotion. The experimental animals were generally less reactive to stress than normal ones. Thus, the author attributed hyperirritability of rats poisoned by DDT to exaggerated motor responses.

The major toxic action of DDT is clearly on the nervous system, and it requires an intact organism for full expression. The fact that DDT causes a myotonic response in muscle and substitution of a train of spikes for the normal diphasic electroneurogram (Eyzaguirre & Lilienthal, 1949) is in marked contrast with the absence of detectable injury or, in fact, any response in other isolated tissues. As early as 1945, Lewis & Richards (1945) found DDT to be inert when it was applied to tissue cultures of heart, kidney, stomach, intestine, liver, and muscle from 7 to 9-day chick embryos, and of brain and spleen from a one-day rat. The physiology of the

cells including the mytoses of fibroblasts was normal. The migration and extension of the various cells was unchanged. The authors stated that "living fibrilloblasts, as they moved about in the cultures, sometimes touched or even migrated over DDT crystals without appreciable injury to themselves during a period of several days". Some observations were carried out for periods as long as 21 days.

In spite of the importance of the nervous system, a detailed review of early literature indicates that, although the presence of some specialized nervous function may be necessary for the manifestation of DDT poisoning, the mere occurrence of specialized nerve fibres in certain protozoa or the occurrence of a rather complex nervous system in molluscs is not sufficient to render these forms susceptible. Just as there is no explanation for the effect of DDT on susceptible species, so there is no explanation for the fact that certain species and even entire phyla are inherently resistant to the compound.

A review (Hayes, 1959) of literature on the effects of DDT on the nervous system reveals that all major parts, both central and peripheral, are affected. Whereas effects on specific portions, notably the cerebellum and the motor cortex, have been viewed as of greatest importance, it probably is more accurate to emphasize the interaction of functions, all modified to some degree.

There is reason to think that the mechanism of action of DDT is its action on membranes in the nervous system, especially axonal membranes. Certainly action on membranes is a fundamental property of the compound. In fact the potassium conductance induced by valinomycin at 10^{-6} mol/litre in a synthetic lecithin-decane membrane is reversed by DDT at 3×10^{-6} mol/litre (Hilton & O'Brien, 1970).

Attention was focused quite early on the effects of DDT on axonal membranes. Using the giant axons of the cockroach, Narahashi & Yamasaki (1960) showed that DDT prolonged the recovery phase of the action potential. They concluded that it slows the efflux of potassium ions from the axon. Later, using the voltage clamp technique and giant axons of the lobster, Narahashi & Haas (1967) showed that DDT, at a concentration of 5×10^{-4} mol/litre of bathing medium, prolonged the flow of sodium ions as well as interfering with the flow of potassioum ions: in other words DDT delayed shutting of the Na^+ gate and prevented full opening of the K^+ gate.

Na^+-, K^+-, and Mg^{2+}-adenosine triphosphatase (EC 3.6.1.3) is involved in ion transport in the nervous system. Matsumura & Patil (1969) showed that a preparation of this enzyme from a nerve ending fraction of the rabbit brain was inhibited by DDT at concentrations as low as 10^{-8} mol/litre. There was a good correlation between the degree of its inhibition by

analogues of DDT and their toxicity to mosquito larvae. A similar enzyme that binds ^{14}C-DDT has been isolated from the synapses of rat brain (Bratowski & Matsumura, 1972). Both the electrophysiological changes and the enzyme inhibition exhibit a negative temperature coefficient, an important feature of DDT poisoning in insects but not in mammals (Hoffman & Lendle, 1948; Deichmann et al., 1950).

At a supralethal dosage of 600 mg/kg, DDT caused a marked decrease in the concentration of cortical and striatal acetylcholine and of brainstem norepinephrine in rats and a significant increase in brainstem 5-hydroxyindoleacetic acid. All of the neurotoxic signs of poisoning were blocked by *p*-chlorophenylalanine, while other inhibitors blocked one or other, but not all of the effects. It was concluded that changes in the metabolism of 5-hydroxytryptamine and norepinephrine might be responsible for DDT-induced hyperthermia while acetylcholine might be related to tremors and convulsions (Hrdina et al., 1973). These and related matters have been reviewed in great detail by Hrdina et al. (1975). Apparently studies have not been made at a range of dosages that would make it possible to know whether these changes are a result or a cause of poisoning; the possible therapeutic effect of *p*-chlorophenylalanine has also not been explored.

It was reported by Haikina & Šilina (1971) that administration of DDT to rats at only one-fifth of the LD_{50} for the 2 days increased the amount of 5-oxyindoleacetic acid excreted in urine by 188%. This indicates a change in the metabolism of serotonin, but its significance is not clear.

One sensitive measure of brain activity is the electroencephalogram (EEG). Farkas et al. (1969) found that the EEG wave frequency increased considerably in resting rats that had received 20 (mg/kg)/day of DDT as a result of dietary intake. Rats that had received either 5 or 10 (mg/kg)/day did not exhibit this change while at rest, but even those receiving 5 (mg/kg)/day exhibited this change when exposed to a rhythmic light stimulus. The EEG may become abnormal only a minute or two after administration of a large dose of DDT; 4 stages of the electrical activity culminating in generalized seizures have been described by Joy (1973). Phenobarbital, but not phenytoin or trimethadione, was effective in stopping the seizures.

7.1.2.1 *Cause of death*

Death from DDT poisoning is usually the result of respiratory arrest. The heart continues to beat to the end and in some instances continues a little while after respiration stops. Deichmann et al. (1950) found that the onset of hyperirritability in rats was accompanied by an increase in the frequency and amplitude of respiration. Later, with the occurrence of tremors, the depth of respiration frequently returned to a more normal level, but the rate

remained high. In some animals, respiration stopped suddenly after a deep inspiration during a tonic convulsion. In other animals, the rate and amplitude decreased progressively and finally ceased without any terminal spasm. Animals that die of respiratory failure caused by DDT do so after a relatively long period of muscular activity that leaves them exhausted.

It was shown by Philips & Gilman (1946) and Philips et al. (1946) that the hearts of dogs given large intravenous doses of DDT were sensitized to epinephrine. This was true not only of injected epinephrine but also of the compound released by the adrenal glands during a seizure. Stimulated in this way, the sensitized hearts of dogs developed an irreversible, fatal ventricular fibrillation. However, the hearts of monkeys were able to recover from fibrillation and resume normal rhythm. It is not clear how important sensitization of the myocardium is when DDT is administered by other routes, but ventricular fibrillation may be the cause of death in animals that die suddenly, soon after onset of poisoning.

7.1.2.2 *Treatment of poisoning in animals*

Studies on the treatment of poisoning will be discussed in this section since all the more successful studies of treatment of animals poisoned by DDT involve the nervous system. Smith and Stohlman (1944) noted the possibility that narcotics in general, may exhibit an antagonism to DDT. Rats survived on a diet containing DDT at a concentration of 1000 mg/kg for 90 days when they received cyclohexanone in the same diet at the rate of 2000 mg/kg, but were uniformly killed in a shorter period when they received DDT at the same rate without cyclohexanone. Later, it was shown that cyclohexanone offers no protection when used as a solvent for single massive doses of DDT (Deichmann et al., 1950).

Smith & Stohlman (1945) showed that, when given as required after the onset of illness, urethane and, to a lesser extent, phenytoin sodium protected rats from poisoning. Sodium amobarbital gave slight benefit, sodium phenobarbital a doubtful benefit, and paraldehyde no protection at all. All drugs were given intraperitoneally except paraldehyde, which was given by stomach tube. The mortality of rats treated with urethane was 12.5% and that of their controls was 80%. A total dosage of 1.2–2.5 mg/kg spread over a period of 1–3 days, was found most satisfactory. Phenytoin sodium gave a mortality of 46.7%, compared with 96.7% for the controls. The smallest effective dosage was 200–250 mg/kg, a value very close to the LD_{50} which, under the conditions of the test was 300 mg/kg.

Lauger et al. (1945a, b) also found that sodium phenobarbital was of questionable value in treating rats poisoned by DDT. However, completely different results were seen in larger animals. Phenobarbital was, by far, the most outstanding remedy tested by Philips & Gilman (1946). In a dosage

well below the anaesthetic level, it not only prevented death in many instances but also controlled tremor and convulsions. Signs of illness were more readily controlled in dogs and cats than in monkeys, which required nearly a full anaesthetic dosage before tremors completely disappeared.

Magnesium sulfate did not reduce mortality in poisoned dogs and cats although it did control tremors and convulsions briefly. Sodium bromide was entirely ineffective. Mortality was reduced with urethane, but a full anaesthetic dosage was required to control tremor and convulsion. Similarly, sodium barbital and sodium pentobarbital controlled symptoms only when given in full anaesthetic doses and, even then, did not greatly reduce mortality. Phenytoin, when given to rats before they received DDT, reduced the lethal action without showing a notable effect on the signs of poisoning; phenytoin was not effective in cats.

Vaz et al. (1945) were, apparently, the first to note the antidotal effect of calcium in DDT poisoning. Dogs were given DDT orally as a 10% oily solution at a daily dosage of 100 mg/kg until signs of intoxication appeared. The same dosage could then be repeated to produce intense symptomatology from which the animals would recover spontaneously in 12–24 h. For the actual tests, a larger challenge dosage of DDT (150 to 200 mg/kg) was used. Each dose of calcium gluconate (30 ml of a 10% solution) was injected intravenously into dogs weighing 8–18 kg. Dogs that were injected with calcium gluconate daily for 4 days and, challenged with a large dose of DDT on the fourth day, did not develop any symptoms or only slight ones. Dogs receiving a single dose of calcium gluconate showed symptoms of short duration and survived following a dosage of DDT large enough to kill 2 controls.

Cats poisoned by the intravenous injection of a soya-lecithin-corn oil emulsion of DDT were studied by Koster (1947). A comparison was made of several aspects of intoxication including number of convulsions, general severity (tremors, prostration, dyspnoea), duration, and mortality. Both calcium gluconate and sodium gluconate reduced mortality but not severity. Gluconic acid increased the survival time, reduced mortality, but did not reduce the number or severity of the convulsions. Calcium chloride reduced convulsions, but not mortality or tremors. Molecular equivalent doses of the candidate antidotes were used. Gluconic acid and its two salts were effective against an LD_{95} dosage of DDT. The life-saving capacity of calcium gluconate at a rate of 40 mg/kg was confirmed by Judah (1949) even though he found normal blood calcium values in most poisoned but unmedicated animals. One animal showed a high calcium value, and Cameron & Burgess (1945) reported a similar result. It has been suggested that increased blood calcium may be associated with acidosis caused by the accumulation of lactate.

Thus, calcium has an antidotal action against DDT in intact animals of several species. The suppression by calcium of the effect of DDT on the isolated nerve and muscle of the rat has been demonstrated (Eyzaguirre & Lilienthal, 1949). The hypothesis has been advanced (Welsh & Gordon, 1946; Gordon & Welsh, 1948) that certain neurotoxins, including DDT, act by delaying the restoration of calcium ions to a surface complex, following breaking of the chelate linkage of calcium ions to surface polar groups by an initial exciting impulse. This action of the neurotoxin is conceived as depending largely on its physical rather than on its chemical properties. The hypothesis is helpful in explaining the fact that a wide variety of chemically unrelated compounds produce repetitive responses in excitable tissue and also the fact that many compounds that show a high toxicity for arthropods and mammals are fat-soluble and relatively inert chemically. It has been pointed out that this hypothesis postulates a very localized action of calcium at the nerve-cell membrane; the hypothesis is not inconsistent with the finding that the blood calcium of poisoned animals may be unchanged or even increased.

Having observed the effect of DDT on the metabolism of glucose and glycogen, Lauger et al. (1945a, b) investigated the use of glucose as an antidote. All of 10 dogs given 2000 mg of DDT per kilogram body weight orally in the form of an oil solution died within 8–24 h. Five of 10 dogs treated with one or more, 20 ml doses of 20% glucose survived the same dosage of DDT. The glucose was given intravenously in most instances.

Koster (1947) found that glucose given before or after an LD_{33} dosage reduced convulsions and mortality and, when given before the poison, reduced tremors, prostration, and dyspnoea in cats. Glucose, unlike gluconic acid and its sodium and calcium salts, was ineffective against an LD_{95} dosage except to increase the time of survival. Insulin, given intramuscularly 16–25 min before DDT, increased the survival time and the severity of poisoning but did not affect mortality or convulsions. When given 53–130 min before DDT, insulin reduced convulsions in animals which died but increased convulsions, tremors, and other disorders in the survivors.

The failure of amino and sulfhydryl compounds to influence the action of DDT was noted by Von Oettingen & Sharpless, 1946). Likewise, the addition of 0.2% choline chloride to the diet of rats receiving repeated doses of DDT had no effect on the accumulation of lipids in their liver (Sarett & Jandorf, 1947).

In summary, it would appear that sedatives, ionic calcium, and glucose or other ready sources of energy are useful in treating poisoning by DDT. Dogs and cats can be treated somewhat more successfully than rats, perhaps because the metabolism of larger animals is slower.

Although respiration may be temporarily restored in animals poisoned by DDT, studies have not been made to determine whether respiratory arrest in this condition is truly reversible as it is in poisoning by certain organic phosphorus insecticides.

7.1.3 Renal system

No dysfunction of the renal system attributable to DDT has been found even in animals receiving dosages sufficient to cause dysfunction of the nervous system or striking morphological changes of the liver. It is true that mild to moderate morphological changes have been reported in the kidneys of animals that have received massive single doses or repeated doses; for example, fatty degeneration, necrosis, and calcification (Lillie et al., 1947; Stohlman & Lillie, 1948) or slight brown pigmentation of the convoluted tubular epithelium (Fitzhugh & Nelson, 1947). However, it sometimes has happened that a complete absence of change in the kidney has been reported in connexion with other studies carried out in the same laboratories (Lillie & Smith, 1944; Nelson et al., 1944).

7.1.4 Gastrointestinal tract, liver, and enzymes

Large doses of DDT produce vomiting in species that can vomit. Only doses that produce rather severe poisoning lead to anorexia.

7.1.4.1 *Liver*

Large doses of DDT cause focal necrosis of liver cells in several species (Lillie & Smith, 1944; Nelson et al., 1944; Cameron & Burgess, 1945; Lillie et al., 1947; Deichmann et al., 1950; Ortega et al., 1956). At least some investigators (Cameron & Burgess, 1945) have considered that the liver lesions produced by large dosages are sufficient to account for death. All have agreed that necrotic lesions occur only in connexion with potentially fatal dosages.

A very different kind of liver change is produced in some rodents but not in other animals by small or moderate dosages of DDT. The biochemical aspects of these changes are discussed in section 7.1.4.2, while the morphological aspects are discussed in section 7.1.9.

7.1.4.2 *Microsomal enzymes of the liver*

All enzymes can be inhibited *in vitro* and many of them can be inhibited *in vivo*. The toxic action of a number of compounds is clearly the result of their inhibition of one or more enzymes. So far, only a few enzymes or enzyme systems are known to be induced by chemicals; the outstanding example is the induction of microsomal, mixed-function enzymes of the liver

and some other organs that are produced in greater quantity in response to certain hormones and other normal constituents of the body (Conney, 1967), some foods (Wattenberg, 1971), or a wide range of drugs or other foreign chemicals. Such induction requires intact cells and does not occur when the inducer is brought in contact with the purified enzymes *in vitro*. Microsomal enzymes were first recognized and studied in the liver, but they are now known to exist, generally in lower concentrations, in other tissues.

Microsomal enzymes are known to be associated with oxidation (*N*-, *O*-, and *S*-dealkylation, deamination, epoxidation, disulfuration, hydroxylation) of both rings and side chains, oxidation of both nitrogen and sulfur, reduction of nitro groups and of azo compounds, hydrolysis, and conjugation. Most of the changes produced by microsomal enzymes render oil-soluble compounds more water-soluble and, therefore, more easily excreted. Mainly for this reason, most biotransformations promoted by microsomal enzymes are true detoxications. However, some of the reactions promoted by this system of enzymes render specific compounds more toxic.

In the rat, DDT has been shown to promote the biotransformation of hexobarbital (Hart & Fouts, 1963), phenazone (Hart & Fouts, 1963; Kinoshita et al., 1966), *p*-nitrobenzoic acid (Hart & Fouts, 1963), aniline (Hart & Fouts, 1963), dieldrin (Street, 1964; Street et al., 1966; Street & Chadwick, 1967; Pearl & Kupfer, 1971), *o*-ethyl *o*-4-nitrophenyl phenylphosphonothioate (EPN) (Kinoshita et al., 1966), *p*-nitroanisole (Kinoshita et al., 1966; Vainio, 1974), methyprylon (Datta & Nelson, 1968), meprobamate (Datta & Nelson, 1968), chlordizepoxide (Datta & Nelson, 1968), aldrin (Gillett, 1968), lindane (Chadwick et al., 1971b), phenylbutazone (Welch & Harrison, 1966), pentobarbital (Fredricks et al., 1974), 3,4-benzpyrene (Vainio, 1974), and *p*-nitrophenol. The biotransformation of *p*-nitrophenol involves conjugation by the microsomal enzyme uridinediphosphoglucuronyltransferase, and its demonstration requires activation of the microsomes, for example by trypsin (Vainio, 1974, 1975; Rantanen et al., 1975).

In the mouse, DDT has been shown to promote the biotransformation of pentobarbital (Gabliks & Maltby-Askari, 1970).

In the guineapig, DDT promoted the metabolism of dieldrin (Wagstaff & Street, 1970).

DDT in the squirrel monkey promoted the metabolism of EPN and *p*-nitroanisole; the first required a DDT dosage of 5.0 (mg/kg)/day, but the latter required only 0.5 (mg/kg)/day (Cranmer et al., 1972). DDT did not promote the metabolism of ^{14}C-DDT in squirrel monkeys (Chadwick et al., 1971a) but did promote the metabolism of phenylbutazone in the dog (Welch & Harrison, 1966) and the metabolism of estradiol in the pigeon (Peakall, 1970).

In the chicken, DDT failed to affect *N*-demethylase or the concentration of cytochrome P-450, and reduced aniline hydroxylase activity. The influences of dosage, duration of dosing species, and reproductive state on microsomal enzymes in birds are poorly understood (Sell et al., 1971).

o,p'-TDE has been shown to promote the metabolism of pentobarbital in the mouse (Gabliks & Maltby-Askari, 1970), phenobarbital in the rat (Straw et al., 1965), and cortisol in the guineapig (Kupfer et al., 1964).

The metabolism of DDT is promoted by DDT itself in the hamster but not in the mouse (Gingell & Wallcave, 1974).

In rats, the metabolism of DDT is promoted by phenytoin (Cranmer, 1970). The metabolism of DDT and DDE in cows is promoted by phenobarbital (Alary et al., 1971; Fries et al., 1971).

DDE, whether fed directly or produced metabolically from DDT, appears to be more important than DDT in inducing microsomal enzymes. The tissue level of DDE necessary for enzyme induction is lower in the rat than in the quail (and presumably other birds). Thus Bunyan et al. (1972), using residues in the heart as an index, found a maximum increase in cytochrome P-450 per gram of liver and a maximum activity of aniline hydroxylase levels at DDE levels of approximately 3 mg/kg in rats and 40 mg/kg in quail. However, at any given dietary level, higher tissue levels were reached by quail than by rats so that the dosage responses of the two were similar.

Different inducers may activate different enzymes and, therefore, different metabolic pathways. Pretreatment of rats with lindane caused them to metabolize a single dose of radioactive lindane 2.5 times more efficiently than controls, whereas pretreatment with DDT caused a 3.5-fold increase in the metabolism of lindane. Furthermore, the DDT pretreatment was followed by a different proportion of radioactive metabolites with a predominance of tetrachlorophenols, especially 2,3,4,5-tetrachlorophenol (Chadwick et al., 1971b).

The enzymes of weanling rats are more subject to induction than those of adult rats, but there is no evidence of a lag-period in induction in adults (Chadwick et al., 1975).

7.1.4.3 *Enzymes of intermediary metabolism*

As discussed in section 7.1.2, there is some reason to think that DDT acts by influencing an enzyme critical to the function of neurones. It is certainly clear that many of the side-effects of DDT are the result of its induction of microsomal enzymes (see sections 7.1.4.2 and 7.1.9). In addition, DDT has been shown *in vitro* and sometimes *in vivo* to influence some enzymes of intermediary metabolism and other miscellaneous enzymes. So far, evidence

is lacking that the degree of this inhibition in the intact organism is sufficient to have any influence on function.

The hyperglycaemia observed during much of the early part of acute poisoning may be associated with an increase in 4 gluconeogenic enzymes (pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxykinase (EC 4.1.1.32), fructose 1,6-diphosphatase, and glucose 6-phosphatase (EC 3.1.3.9)). Increases in these enzymes in the renal cortex of rats, observed after a single dose at a rate as low as 100 mg/kg, were greater at a dose of 600 mg/kg. The reaction occurred in both adrenalectomized and normal rats (Kacew & Singhal, 1972). Similar responses were observed in the same enzymes in the liver following a single dose at the rate of 100 mg/kg or more or following 45 daily doses at rates of 5 or 25 (mg/kg)/day. The changes are not mediated through a release of corticosteroids from the adrenal glands (Kacew & Singhal, 1973). The same authors (Hrdina et al., 1975; Singhal & Kacew, 1976) have reviewed the extensive evidence contributed mainly by themselves indicating that the changes in glucose homeostasis are mediated by stimulation of the cyclic adenosine monophosphate (AMP)-adenylate cyclase system in the liver and in the kidney cortex by *p,p'*-DDT and a number of other organic chlorine pesticides. However, the smallest single dosage of *p,p'*-DDT that produced a statistically significant change in the enzymes or in the production of cyclic AMP was 180 mg/kg and *o,p'*-DDT was as effective as the *p,p'*-isomer; these findings indicate that the carbohydrate changes are results not causes of poisoning.

DDT and some other compounds induce increased activity of D-glucuronolacetone dehydrogenase (EC 1.1.1.70) in the supernatant fraction of rat liver, and this is consistent with evidence of increased D-glucaric acid in urine after dog treatment (Marselos & Hanninen, 1974). Urinary excretion of L-ascorbic acid is also increased by DDT, but apparently this is not caused by an increase in an enzyme producing this acid but rather by an increase in several microsomal and cytosol enzymes that contribute to an increase in free glucuronic acid from which L-ascorbic acid is formed (Rantanen et al., 1975).

A review (Hayes, 1959) of early literature indicates that high concentrations of DDT inhibit phosphatidase (EC 3.1.1.4), muscle phosphatases, carbonic anhydrase (EC 4.2.1.1), oxalacetic carboxylase (EC 4.1.1.3), and increase the activity of cytochrome oxidase (EC 1.9.3.1) and succinic dehydrogenase (EC 1.3.99.1). However, none of these changes with the possible exception of inhibition of carbonic anhydrase could be shown to have any connexion with the toxic action of DDT or even with its side-effects. Neal et al. (1944) reported a small but consistent increase in the volume of urine excreted in 24 h when dogs were dosed orally or by insufflation at the rate of 100 (mg/kg)/day. No other change in the urine

and no change in kidney function was demonstrated. The possibility that increased urinary output is related to the inhibition of carbonic anhydrase (Torda & Wolff, 1949) may deserve attention. However, re-examination of data from volunteers receiving 3.5 or 35 mg/man per day did not indicate any increase in urinary volume compared with controls (Hayes et al., 1971).

It has been claimed (Keller, 1952) that DDT inhibits carbonic anhydrase in bovine erthrocytes. However, the method was criticized by Dvorchik et al. (1971), who found *in vitro* inhibition only by concentrations of DDT, unlikely to be survived by living animals. Far more attention has been given to inhibition of carbonic anhydrase in the shell gland of birds than in erythrocytes, and it has been suggested (Bitman et al., 1970; Peakall, 1970) that inhibition of the shell gland enzme is an explanation for eggshell thinning in certain birds. However, the same criticism holds for the shell gland; there is no evidence that the degree of inhibition reported interferes with function.

On the other hand, many enzymes including plasma amylase, aldolase (EC 4.1.2.13), glutamic-pyruvic transaminase (EC 2.6.1.2), and isocitric dehydrogenase (EC 1.1.1.41) were not changed in squirrel monkeys given dosages ranging from 0.05 to 50 (mg/kg)/day; the highest dosage proved fatal within 14 weeks (Cranmer et al., 1972).

7.1.5 Cardiovascular system

Most dogs, killed by a single dose of DDT, die of ventricular fibrillation, and the same is true of some cats, monkeys, and rabbits (Philips & Gilman, 1946). At any given dosage of DDT, ventricular fibrillation is more likely to occur if the animal receives exogenous epinephrine or is stimulated in such a way that the compound is released by the adrenals. Besides fibrillation, dogs may exhibit extrasystoles and changes in the T-wave (Philips et al., 1946). Monkeys differ from dogs in that the DDT-sensitized heart is able to recover from fibrillation and resume a normal rhythm (Philips et al., 1946).

Thus, DDT not only sensitizes the myocardium in a way similar to that of halogenated hydrocarbon solvents, but, through its action on the central nervous system, produces the stimulus that increases the likelihood of fibrillation.

There is no evidence that repeated, tolerated doses of DDT sensitize the heart. Rats were fed DDT at a dietary level of about 10 (mg/kg)/day for 8 months, during which time they received weekly, intraperitoneal doses of vasopressin, a compound that causes a temporary myocardial ischaemia. Electrocardiograms did not show any significant increase in cardiac arrhythmias in the DDT-fed rats compared with controls. Intravenous noradrenalin given at the end of the 8-month period did not produce a

greater incidence of arrhythmias in the DDT-fed rats. The same results were obtained in rabbits treated in essentially the same way (Jeyaratnam & Forshaw, 1974).

TDE. The main action of TDE, and especially of *o,p'*-TDE, is on the liver with secondary effects on the adrenals in dogs and perhaps in other species. However, Cueto (1970) showed that at a dosage of 50 (mg/kg)/day for 14 days, *o,p'*-TDE caused a gradually progressive hypotensive failure in dogs injected with epinephrine or norepinephrine, while leaving the cardio-accelerator and immediate pressor response to these drugs unchanged. The hypotensive failure was associated with weakening of the contractile force of the heart and with a reduction of plasma volume. The latter may have been caused by a loss of fluid from the intravascular compartment and was not caused by a release of histamine. The hypotensive state could be prevented to a significant degree by pretreatment with prednisolone.

7.1.6 Respiratory system

The effects of DDT on the respiratory system are secondary to effects on the nervous system and are discussed in section 7.1.2.1.

7.1.7 Reproductive system

It was shown very early (Burlington & Linderman, 1950) that DDT produces a striking inhibition of testicular growth and secondary sexual characteristics of cockerels, when injected subcutaneously in dosages as high as 300 (mg/kg)/day. Changes in the testis involve the tubules, and not the interstitial tissues, and they have been attributed to an estrogen-like action of DDT.

It must be noted that the action of DDT on the testis of the chicken is dosage-related. Before the problem of residues became evident, DDT was used extensively for control of lice and common mites on chickens without any adverse effects on egg production or other aspects of reproduction. Many rats would be killed the first day if they were given the dosage of DDT that has been shown to affect the testis in cockerels. The report that, under special conditions, DDT has a gonadotoxic effect (Rybakova, 1968) is of questionable significance in view of the results of multigeneration tests in rats, mice, and dogs.

Ottoboni (1969) found that female rats reproduced normally when fed DDT for two generations at dietary levels as high as 200 mg/kg (about 10 (mg/kg)/day, except during lactation when intake was increased about 3-fold). In fact, at a dietary level of 20 mg/kg, the dams had a significantly longer reproductive life span (14.55 months) than did their littermate

controls (8.91 months); the number of females becoming pregnant after the age of 17 months and the number of successful pregnancies after that age differed significantly between the two groups (Ottoboni, 1972).

In a study focused mainly on DDT in milk, the ability of rats to reproduce at a dietary level of 200 mg/kg was confirmed, and the ability of dams, injected intraperitoneally at levels as high as 100 (mg/kg)/day, to rear their young was demonstrated (Hayes, 1976b).

A six-generation test of reproduction in mice did not show any effect of DDT at a dietary level of 25 mg/kg on fertility, gestation, viability, lactation, and survival. A dietary level of 100 mg/kg produced a slight reduction in lactation and survival in some generations but not all, and the effect was not progressive. A level of 250 mg/kg was distinctly injurious to reproduction (Keplinger et al., 1970). The dietary concentrations used equalled dosages of 3.33, 13.3 and 33.2 (mg/kg)/day in nonpregnant, nonlactating, adult female mice. The intake is much higher in both young and lactating mice. The authors concluded that their study provided no obvious reason for continuing reproduction tests for more than three generations.

Four female dogs of unstated age that had previously received DDT at the rate of 12 (mg/kg)/day, 5 days a week, for 14 months were mated when they went into heat. The males involved had been fed aldrin (0.15 (mg/kg)/day) plus DDT (60(mg/kg)/day) for 14 months prior to breeding but not during breeding. Two of the females went into heat but failed to become pregnant, and one failed to come into heat during 12 months after feeding stopped. Four of 6 pups born to the fourth female died within one week of birth; the other 2 were weaned successfully even though only 2 posterior mammae of the mother were functional (Deichmann et al., 1971b). A 3-generation study failed to confirm any of the injuries suggested by the study of 4 dogs. In the 3-generation study, male and female dogs were fed technical DDT from weaning at rates of 0, 1, 5, and 10 (mg/kg)/day. Observations were made on 135 adult females, 63 adult males and 650 pups. There were no statistically significant differences between controls and DDT-treated dogs in length of gestation, fertility, success of pregnancy, litter size, lactation ability of the dams, viability at birth, survival to weaning, sex distribution, growth of pups, morbidity, mortality, organ/body weight ratios, or gross histological abnormalities in all the animals studied. The only clear difference was that DDT-treated females had their first estrous 2 or 3 months earlier than the control animals. There was a slight increase in liver/body weight ratio in some DDT-treated animals but the difference was not statistically significant, dosage related, or associated with any histological change (Ottoboni et al., 1977).

o,p'-DDT. Intraperitoneal injection of technical DDT at a dosage as low as 5 mg/kg or of *o,p'*-DDT at 1 mg/kg caused a significant increase in

the weight of the uterus of normal, immature female rats or of ovariectomized adult females. Stimulation caused by *p,p'*-DDT was much less. Treatment of rats with DDT, especially *o,p'*-DDT, 2 h before injection of estradiol-17-6,7-^{3}H inhibited uptake of the hormone by the uterus *in vivo*, possibly by competition for binding sites. Isomers of TDE and DDE do not influence uterine weight or the binding of estradiol (Welch et al., 1969). It seems unlikely that metabolic activation of *o,p'*-DDT is necessary, as is true of *o,p'*-methoxychlor. The binding and estrogenic activity of DDT analogues in rats is only about 1/10 000 as great as that of diethylstilbesterol (Nelson, 1973).

A considerably smaller dosage of *o,p'*-DDT resulting from a dietary level of 10 mg/kg for 2–9 months did not have any effect on reproduction in ewes (Wrenn et al., 1971b). In a similar way, dietary levels of *o,p'*-DDT as high as 40 mg/kg, giving a dosage level of about 2.1 (mg/kg)/day in rats, failed to interfere with reproduction and lactation in these animals although dosage was continued throughout 2 pregnancies (Wrenn et al., 1971a).

The report (Heinrichs et al., 1971) that *o,p'*-DDT significantly advances puberty, induces persistent vaginal estrus after a period of normal estrus cycles, and causes other reproductive abnormalities in female rats would appear at first to be inconsistent with the lack of effect of technical DDT or of *o,p'*-DDT on reproduction cited above. The same is true of other effects of *o,p'*-DDT demonstrated by the same investigators (Gellert et al., 1972). The abnormal effects were obtained initially by injecting 1 mg of the *o,p'*-DDT subcutaneously on the second, third, and fourth days of life (counting the day of birth as zero). Because rat pups on the third day weigh about 12 g or less each, it follows that the subcutaneous dosage was about 83.3 (mg/kg)/day or more, that is about 40 times greater than the highest oral dosage of *o,p'*-isomer fed to breeding rats and about 10^5 times greater than the levels ingested by the general population with their food.

Because of its estrogenic properties, DDT was considered as a possible cause of abortion in dairy cattle, but no evidence of a relationship was found (Macklin & Bibelin, 1971).

7.1.8 Endocrine organs

Except for the weak estrogenic properties of *o,p'*-DDT, the endocrine-related effects of DDT and its analogues are confined to the adrenals, and even these effects of 2 isomers of TDE are now considered to be mainly secondary to the induction of microsomal enzymes of the liver in most species.

TDE. TDE (DDD) is an insecticide in its own right as well as a metabolite of DDT. The compound is used as a drug to control different

forms of adrenal overproduction of corticoids in man (see section 8.2.8). This therapy was originally based on the demonstration that DDD (Nelson & Woodard, 1948, 1949) and especially *o,p'*-TDE (Cueto & Brown, 1958) caused gross atrophy of the adrenals and degeneration of the cells of its inner cortex in dogs. This is true even though it was originally reported (Nelson & Woodard, 1948, 1949) that TDE produced almost no detectable damage to the adrenals of rats, mice, rabbits, and monkeys, and the finding was confirmed and extended by other investigators to other species, including man (Zimmerman et al., 1956). In the dog, *o,p'*-TDE produced gross atrophy of the adrenals, when administered at a dosage of only 4 (mg/kg)/day. The dosage of technical grade TDE required to produce the same effect was 50–200 (mg/kg)/day (Cueto & Brown, 1958). However, in spite of its exceptional susceptibility, there is a definite threshold below which the dog does not respond. About 15% of technical DDT is *o,p'*-isomer, much of which is gradually metabolized to *o,p'*-TDE (*o,p'*-DDD). Yet dogs remained healthy and reproduced normally in a 3-generation study involving dosages of technical DDT as high as 10 (mg/kg)/day (see section 7.1.7).

It has recently been shown that, following massive dosage (60 mg/kg, administered intravenously), all of the isomers of TDE inhibited ACTH-induced steroid production in the dog, but the inhibition reached 50% of the control only 27 min after dosing with the *m,p'*-isomer compared with 87 min with the *o,p'*-isomer and 4–18 h with the *p,p'*-isomer. There was marked temporal correlation between the percentage inhibition of adenocorticotropic hormone (ACTH)-induced steroid production, the disruption of normal cellular structure of the fascicular and reticular zones of the adrenal cortex, and the severity of the damage to mitochondria in these zones caused by the 3 isomers (Hart et al., 1973). The effectiveness of *m,p'*-TDE for treating metastatic adrenocortical carcinoma had already been demonstrated (Nichols et al., 1961), but it cannot be said that its value for this purpose has been compared adequately with that of *o,p'*-TDE. Furthermore, no effort has apparently been made to compare the effect of small daily doses of the 2 isomers in dogs.

Like other organochlorine insecticides, *o,p'*-TDE stimulates hepatic microsomal oxygenation of both drugs and steroids and, according to a very thorough review by Kupfer (1967), this may explain much of its action on corticoid metabolism in a wide range of species. Increased breakdown is indicated by increased excretion of polar metabolites, while nonpolar metabolites remain stable or even decrease—a finding recently encountered in human patients (Hellman et al., 1973). However, the demonstrated effect on corticoid metabolism fails to explain why *o,p'*- and *m,p'*-TDE are unique in their overall effects on the adrenals, including their ability to produce

adrenocortical atrophy in the dog. Other powerful inducers of microsomal enzymes lack these effects. It is clear that a reduction of steroid production accompanies atrophy of the adrenals of the dog. The review already cited (Kupfer, 1967) considers: (*a*) reduced steroid production in species other than the dog, including the possibility that such reduction is secondary to inhibition of glucose-6-phosphate-dehydrogenase (EC 1.1.1.49) activity in the adrenals and (*b*) blockage of steroid action by a steroid metabolite formed under the influence of DDD. However, the existence of these effects, much less their importance, remains obscure.

o,p'-DDT. Oral administration of *o,p'*-DDT to dogs at a rate of 50 (mg/kg)/day stimulated the microsomal enzymes of the liver as indicated by increase in liver size, total protein, microsomal protein, and cytochrome P-450 concentration and by direct measurements of enzyme activity. These changes in the liver were accompanied, initially, by an increase in the size of the adrenals and of the cells of the zona fasciculata; these cells became vacuolated and devoid of acidophilic cytoplasm, and their nuclei became hyperchromatic and often peripheral in position. Synthesis of corticosteroids by the adrenal was not blocked (Copeland & Cranmer, 1974). Thus the effect of a substantial dosage of *o,p'*-DDT was quite different from that of *o,p'*-TDE (DDE), although part of the metabolism of *o,p'*-DDT must be by the same route.

7.1.9 Carcinogenicity

There is no doubt that DDT and a number of other organochlorine insecticides cause marked changes in the liver in various rodents and that these changes progress to tumour formation in some species, notably the mouse. There is serious disagreement as to whether the mouse tumours are malignant. Regardless of their nature, there is virtual certainty that they are peculiar to rodents and, therefore, interpretation of their significance for man or useful animals is difficult.

Evidence for the carcinogenicity of DDT and its metabolites has been reviewed by the International Agency for Research on Cancer (IARC, 1974). Most of the experimental results are summarized in Table 17. The conclusions of the IARC were that: (*a*) the hepatocarcinogenicity of DDT administered by the oral route has been demonstrated in several strains of mice, and shows a dosage-response relationship; (*b*) a dietary level of 2 mg/kg (above 0.3 (mg/kg)/day) produces a significant increase in hepatomas in male but not in female CFl mice and not in either sex of BALB/c mice; (*c*) increased incidence of tumours has been reported in some other organs of mice but not confirmed in multigeneration studies using a wide range of dosages; (*d*) evidence for the carcinogenicity of DDT in rats

is not convincing and is negative in hamsters even at the higher dietary levels that they tolerate in comparison with rats and mice; (*e*) negative results in dogs and monkeys are inconclusive because of the small groups studied and the short duration of treatment; (*f*) liver cell tumour induction in trout is inconclusive because of a lack in control of the diet; and (*g*) the carcinogenicity of *p,p'*-DDE is similar to that of DDT, but TDE produces a significant incidence of lung tumours.

Actually, the number of dogs and monkeys was not small compared with similar studies on other chemicals. In an investigation on monkeys, dosing at lower levels was continued for 7.5 years. In both dogs and monkeys, dosages sufficient to cause liver damage, death, and neurological indications of DDT poisoning were included in the protocols (see Table 17). Apparently no new studies on dogs and monkeys have been reported. On the other hand, a new study on rats (Rossi et al., 1977) has given definite evidence of the tumorigenicity of DDT and phenobarbital, confirming the conclusion of Fitzhugh & Nelson (1947) which was based on less extensive data.

In mice, liver tumours similar to those caused by DDT (see Table 17) have been reported in connexion with DDE and DDD (Tomatis & Turusov, 1975), chlorobenzilate, HCH, aldrin, dieldrin, mirex, and terpene polychlorinates (Tomatis et al., 1973). Similar tumours have been caused by the important drug, phenobarbital (Wright et al., 1972; Peraino et al., 1973; Thorpe & Walker, 1973; Ponomarkov et al., 1976). TDE also caused lung tumours (Tomatis et al., 1974a).

The IARC review did not discuss the controversy over the nature of the tumours induced by DDT in the livers of some rodents, and it did not consider the relationship of these tumours to the induction of microsomal enzymes. The following paragraphs are concerned with these matters and specifically with those inducers that behave like DDT. The biochemical pattern of induction of mixed function oxidase enzymes is similar for DDT and phenobarbital but distinctly different for 3-methylcholanthrene (Vainio, 1975). Thus 3-methylcholanthrene and compounds like it must be excluded from the discussion. Similarly, the high degree of correlation between the ability of compounds to induce parenchymal liver tumours in mice and their ability to induce tumours in the liver and other organs of rats and hamsters cannot be accepted uncritically. As demonstrated in a study by Tomatis et al. (1973), this correlation is extremely good for compounds that are, or are suspected of being, carcinogens in man. However, the correlation is poor for organochlorine insecticides. In fact, the only one of these compounds that has increased the incidence of a tumour in another species is DDT, which induced liver tumours in rats in one experiment.

The early morphological response of the rodent liver to DDT is similar to

Table 17. Effect on various animals of repeated oral doses of DDT

Dosage		Species	Duration	Results			Reference
Range (mg/kg)/day	Method and concentration			Animals per test (No.)	Mortality (%)	Other	
41–80	800 mg/kg in diet	rat[a]	2 years	36 males, 24 females		Increased mortality, typical liver changes, liver tumours	Fitzhugh & Nelson, 1947
	46 mg/kg then 140 mg/kg in diet	mouse[b]	18 months	36 males, 36 females		Hepatomas in 51 and 21% of males and females respectively compared with 18 and 0.6% of controls	Innes et al., 1969
	3200 mg/kg in diet	dog	39–49 months	10	100	Liver damage, no tumours	Lehman, 1965
	5000 mg/kg in diet	monkey	70 days	1 male	100	Fatal poisoning	Durham et al., 1963
21–40	400 mg/kg in diet	rat[a]	2 years	24 males, 12 females		Increased mortality, typical liver changes	Fitzhugh & Nelson, 1947
	500 mg/kg in diet	rat	2.9 years	37 males, 35 females		Liver tumours in 45%	Rossi et al., 1977
	250 mg/kg in diet	mouse[c]	2 generations	103 males, 90 females		Risk of liver tumour increased 3.7 and 18.5 times in males and females, respectively	Tomatis et al., 1972
	250mg/kg in diet	mouse[c]	2 generations	31 males, 121 females		Liver tumours in 48 and 59% of males and females, respectively	Terracini et al., 1973
	2000 mg/kg in diet	dog	39–49 months	4	25	Minor liver damage but no tumours	Lehman, 1965
11–20	100 mg/kg in diet	mouse[d]	2 years	100 males, 100 females		Hepatomas increased in females of one strain but no increase in hepatocarcinomas	Fitzhugh, 1970
	100 mg/kg in diet	mouse[c]	2 years	30 males, 30 females		Risk of liver tumours increased 4.4 times	Walker et al., 1973
	100 mg/kg in diet	mouse[c]	2 years	30 males, 3 females		Risk of liver tumours increased 3.3 and 4.2 times in males and females respectively	Thorpe & Walker, 1973

Table 17—*continued*

5–10	50 mg/kg in diet	mouse[c]	2 generations	127 males, 104 females	Risk of liver tumours increased 2.45 and 3.46 times in males and females, respectively	Tomatis et al., 1972
	50 mg/kg in diet	mouse[c]	2 years	30 males, 30 females	Risk of liver tumours increased 2.9 times	Walker et al., 1973
	400 mg/kg in diet	dog	39–49 months	2 0	No effect	Lehman, 1965
2.6–5	20 mg/kg in diet	mouse[e]	2 generations	48 males, 128 females	No increase in tumours	Terracini et al., 1973
	200 mg/kg in diet	monkey	3–7.5 years	5 males, 5 females	No toxic effect	Durham et al., 1963
1.26–2.5	10 mg/kg in diet	mouse[c]	2 generations	104 males, 124 females	Risk of liver tumour increased 2.26 and 2.46 times in males and females, respectively	Tomatis et al., 1972
0.626–1.25	25 mg/kg in diet	rat	2 years		No clinical effect; males survived longer than controls	Treon & Cleveland, 1955
	50 mg/kg in diet	monkey	1.6 years	4 males, 1 female	No toxic effect	Durham et al., 1963
0.3126–0.625	10 mg/kg in diet	rat	2 years		Typical liver changes; no effect on reproduction	Fitzhugh, 1948
	12.5 mg/kg in diet	rat	2 years		No effect	Treon & Cleveland, 1955
	2.8–3.0 mg/kg in diet	mouse[e]	5 generations	683	Tumours in 28.7% in controls[f]	Tarjan & Kemeny, 1969
0.15626–0.3125	2 mg/kg in diet	mouse[e]	2 generations	124 males, 111 females	Risk of liver tumour doubled in males, unchanged in females	Tomatis et al., 1972
	2 mg/kg in diet	mouse[e]	2 generations	58 males, 135 females	No increase in tumours	Terracini et al., 1973
0.078126–0.15625	2.5 mg/kg in diet	rat	2 years		No effect	Treon & Cleveland, 1955
	5 mg/kg in diet	monkey	1.4–7.5 years	5 males	No toxic effect	Durham et al., 1963

[a] Osborne-Mendel.
[b] Both (C57BL/6 × C3H/Anf) F1 and (C57BL/6 × AKR) F1 mice.
[c] CFI mice.
[d] BALB/cJ and C3HeB/Fe J.
[e] BALB/c mice.
[f] Lung carcinoma in 16.9% to 1.2% in controls; lymphomas 4.8% compared to 1.0% in controls; leukaemias 12.4% and 2.6%; other tumours 5.8% and 1.0%, respectively.

its response to moderate dosages of HCH, chlordane, dieldrin, camphechlor (Toxaphene) (Lehman, 1952; Ortega et al., 1956), and the important drug, phenobarbital (Wright et al., 1972; Thorpe & Walker, 1973). The earliest change involves so much increase in the smooth endoplasmic reticulum of individual liver cells that they enlarge, and the large granules that are ordinarily scattered throughout the cytoplasm are displaced to the periphery of the affected cell. Quite early, some of the endoplasmic reticulum forms whorls that may have fat droplets as their centres—thus justifying the term "lipospheres", applied to them by Ortega et al. (1956). Others have referred to these inclusions as "hyaline oxyphil masses" (Lillie & Smith, 1944) or "lamellar bodies" (Ito et al., 1973). These changes are accompanied by some increase in fat droplets, not all of which become surrounded by endoplasmic reticulum. This combination of changes (hypertrophy, margination, and lipospheres) is characteristic of rodents and of compounds that induce microsomal enzymes. Certain other changes have been reported but not confirmed. These include enlargement and morphological changes of the mitochondria (Obuchowska & Pawlowska-Tochman, 1973; Watari, 1973), increased numbers of primary lysosomes, and atrophy of the Golgi body (Watari, 1973), none of which were found by Ortega (1966).

The characteristic changes develop promptly. An increase in smooth endoplasmic reticulum and the appearance of lamellar structures have been seen as early as 4 and 7 days, respectively, after the beginning of dosing (Wright et al., 1972).

Although microsomal enzymes may be induced in other species, morphological changes in the liver, as viewed by the light microscope are not the same (Laug et al., 1950; Lehman, 1952; Ortega et al., 1956), or occur to lesser degree as viewed by the electron microscope (Wright et al., 1972).

The changes in liver cells that characterize the induction of microsomal enzymes in rodents are distinct from the focal necrosis that may be produced with about the same ease in the livers of rodents or other species by fatal or near fatal dosages of organochlorine insecticides. The necrotic lesions do not progress because, if such high dosages are continued the animal dies, and, if dosing is stopped and the animal survives, the necrotic cells are removed by autolysis and phagocytic action and the lesions usually heal without scarring (Cameron & Burgess, 1945). Nevertheless, some scarring was found by Lillie & Smith (1944) and Lillie et al. (1947).

At least in the early stages, the changes in liver cells that characterize induction of microsomal enzymes in rodents are reversible (Fitzhugh & Nelson, 1947; Ortega et al., 1956; Wright et al., 1972). The reversibility does not depend on cell removal but simply on reversion of the physiological and morphological condition of the cells to their original condition.

Of course, reversibility is incompatible with progression, but whether

observed irreversibility will be associated with progression must be determined directly in each instance. In the following paragraphs, the question of progression is discussed only after consideration of the problem of irreversibility in general.

If dosing with organochlorine insecticides or other inducers is continued long enough and at a sufficiently high level, the liver changes become irreversible, if for no other reason than that the remaining life span of the animals is too short to permit excretion of the inducing chemical or complete reversion of the liver cells to their original state. The stage at which this shift to irreversibility occurs remains unknown, but it seems very likely that dosages sufficient to produce irreversible morphological change also exceed the physiological adaptability of the liver. The important distinction between adaptation and injury in relation to enzyme induction has been studied using dieldrin.

Although some persons have tended to view even moderate enlargement of the liver or of individual liver cells as an injury, the evidence is strong that these changes are usually adaptive and beneficial to the organism, when they are the result of an increase of smooth endoplasmic reticulum and an associated increase in the activity of liver microsomal enzymes (Barka & Popper, 1967). However, it is obvious that any stimulus or effect may be harmful, if excessive. Hutterer et al. (1968) demonstrated that a distinction may be drawn between adaptation to dieldrin and decompensation resulting from excessive doses of it. Some of these authors, including Popper, showed that the same distinction could be drawn in connexion with other sources of potential liver injury.

It was found that daily intraperitoneal administration of dieldrin to rats at the rate of 2 mg/kg produced enlargement of the liver, hypertrophy of the smooth endoplasmic reticulum, increases in microsomal protein and P-450 haemoprotein, and associated increases in the activity of microsomal enzymes; however, normal activity of other enzymes not derived from the microsomes was maintained. The activity of microsomal enzymes per mole of available P-450 haemoprotein remained unchanged. The highest level of activity of the processing enzymes was reached after 14 days, after which the new steady state was maintained. Rats that had received dieldrin at a rate of 2 mg/kg per day for 28 days were more tolerant to dieldrin than normal rats, as shown by the fact that they survived 25 consecutive daily doses at the rate of 5 mg/kg, a dosage that produced 70% mortality in previously untreated rats. In spite of the ability of the rats, pretreated with a moderate dosage of dieldrin, to survive a large dosage, their livers showed definite indications of decompensation in response to the high dosage. Although the smooth endoplasmic reticulum remained hypertrophic and the microsomal protein and P-450 haemoprotein concen-

trations remained elevated, the enzyme activities per mole of available P-450 haemoprotein decreased, as did the activity of some enzymes not associated with microsomes. Much of the excess smooth endoplasmic reticulum formed tightly packed clusters of tubular membranes with no glycogen and little hyaloplasm, and some of the mitochondrial membranes were injured. It was suggested that the phase of decompensation represented by hypertrophic but hypoactive smooth endoplasmic reticulum might serve as a sensitive criterion of toxic injury before microscopic changes of a clearly harmful sort become recognizable (Hutterer et al., 1968).

Other studies indicating not only the presence of adaptive change over a range of dosages but also the failure of adaptation and onset of injury at sufficiently high dosage levels have been reported for butylated hydroxytoluene (Gilbert & Golberg, 1967) and for DDT (Hoffman et al., 1970). Hoffman and his colleagues found that, when DDT was fed to male weanling rats for only 14 days at dietary concentrations of 0.5 to 2048 mg/kg, concentrations of 0.5 and 2 mg/kg had no effect on the *O*-demethylation reaction used as a test, but concentrations of 4–750 mg/kg produced increases in the rate of metabolism, proportional to the log of dosage. Extrapolation of this portion of the dosage response curve to the abscissa provided a calculated no-effect level of 3.27 mg/kg equivalent to about 0.327 (mg/kg)/day. This is in reasonable agreement with other estimates of the threshold for induction of various enzymes in the rat, including some studies involving longer administration of DDT. These estimates, expressed as (mg/kg)/day, are approximately 0.05 (Kinoshita et al., 1966; Street et al., 1969), 0.5 (Schwabe & Wendling, 1967) and 0.125 (Gillett, 1968). All of the estimates are of the same order of magnitude as the 0.25 (mg/kg)/day known to be effective in man (Laws et al., 1967; Poland et al., 1970), but all are over 100 times greater than the highest dosage received by members of the general population during the late 1960s (Duggan, 1968). Increasing the dietary level to more than 750 mg/kg did not produce any further increase in enzyme activity. Intake of less than 128 mg/kg did not produce any increase in liver weight, within the period of observation; increase was proportional to dosage within the range of 128 to 512 mg/kg and was submaximal at intakes above 512 mg/kg.

Some other compounds, notably phenobarbital, produced morphological changes in the liver similar to those produced by some organochlorine insecticides (Wright et al., 1972; Thorpe & Walker, 1973). It seems possible that sufficiently high doses of phenobarbital, for example, may lead to a failure of adaptation and to levels of enzyme activity that do not correspond to dosage.

As indicated above, the earliest morphological changes caused by enzyme inducers in the rodent liver involve separate cells in the centrolobular area. If

the dosage is sufficiently high and prolonged, nodules consisting entirely of hypertrophied cells may appear. At first, these microscopic nodules are distinguishable only by pattern; they have no bounding membrane and they do not compress or change in any other detectable way the smaller liver cells that surround them. Some nodules may become large enough to be seen without a microscope, and a few may exceed 1 cm in diameter. In these large nodules, there is almost complete loss of lobular architecture. Nodules apparently were first described by Fitzhugh & Nelson (1947) who felt that they could be regarded as adenomas or as low grade hepatic cell carcinomas. The use of the second term is not clear because neither mitoses, tissue invasion, nor metastasis was observed. Although Ortega et al. (1956) reported small nodules in the livers of treated rats, and although they examined tissue loaned by Fitzhugh & Nelson's laboratory, they were entirely unimpressed by the lesions, referring to them as "focal incongruities".

Almost 3 decades after the first study, there is no more agreement than is reflected in the preceding paragraph. The views of some pathologists remain diametrically opposed. This is true even though the finding of: (*a*) pulmonary metastases of hepatic cells in mice that had received DDT (Tomatis et al., 1972; Walker et al., 1973), β-HCH, γ-HCH, dieldrin, or phenobarbital (Thorpe & Walker, 1973); or (*b*) progression of liver enlargement beginning 12 weeks after cessation of ingestion of α-HCH by mice for 24 or 36 weeks (Nagasaki et al., 1974) or progressive increase in the size of liver nodules after DDT feeding was stopped (Tomatis et al., 1974b; Tomatis & Turusov, 1975); or even (*c*) in HCH-exposed mice the time-pattern of increase in liver weight (as reflected by body weight), which gained momentum only after a delay of 4 weeks but showed a further acceleration in the thirteenth week, in spite of decreased food consumption (Tomii et al., 1972), would appear to establish, without question, that at least some of the liver changes produced by these compounds in rodents are malignant.

Of course, the reason for disagreement is that the tumours produced by DDT, other organochlorine insecticides, and phenobarbital differ in their biochemistry and are not malignant in the classical sense. Specifically, (*a*) they do not actively invade tissues; (*b*) their "metastases" do not grow; (*c*) they produce little shortening of life span; and (*d*) mice receiving 5.5 (mg/kg)/day as a result of dietary intake of DDT show a decrease in the success of transplantation and a significant increase in survival in mice in which tumours grew following inoculation with an otherwise uniformly transplantable and uniformly fatal ependymoma (Laws, 1971).

Although the displacement of liver cells to the lung occasionally seen after prolonged dosage with DDT is usually referred to as metastasis, it might better be called embolism because the lesion does not progress and, there-

fore, lacks the clinical significance of a real metastasis. Because it does not grow, the lesion is hard to find. A number of investigators have failed to mention liver cells in the spleen, lymph nodes, or lungs, and some have stated specifically that they were not found (Nagasaki, 1973; Rossi et al., 1977).

Perhaps the most illuminating study of the liver changes caused by DDT is that of Kuwabara & Takayama (1974). They used fluorinyl acetamide (2,7-FAA) as a positive control in their studies of DDT and HCH. The 3 compounds were given at dietary concentrations of 250, 250, and 600 mg/kg, respectively. The lesion caused by 2,7-FAA differed from those caused by either of the other compounds in 3 ways: (*a*) it started as hyperplastic nodules rather than as isolated cell changes; (*b*) the final lesion was hepatocellular carcinoma in contrast with the adenoma caused by DDT or HCH; and (*c*) α-fetoprotein was formed, which did not occur with DDT or HCH. Other workers have also failed to find α-fetoprotein in mice treated with an organochlorine insecticide (Hanada et al., 1973).

It must be emphasized that the organochlorine insecticides and phenobarbital do not produce, in other animals, the early, visible changes in the endoplasmic reticulum that are so characteristic of some rodents and that progress to tumour formation in rodents. That these compounds do not lead to tumour formation in other animals might have been predicted by the fact that they do not cause the early changes, characterized by hypertrophy, margination, and lipospheres.

All available evidence indicates that man does not appear to be susceptible to the tumorigenic action of the organochlorine insecticides and phenobarbital. No increase in the occurrence of tumours has been found in heavily-exposed populations. This includes groups of workers who manufacture and formulate DDT and dieldrin and who have been examined carefully for tumours (Laws et al., 1967; Jager, 1970).

Finally, a study based on a complete tumour registry did not indicate any increase of tumours attributable to phenobarbital among men and women who received heavy, essentially lifelong dosing with this drug for the control of epilepsy (Clemmesen et al., 1974).

In summary, in spite of disagreement about the interpretation of the liver cell changes, there is general agreement about their development and appearance. The change that can be detected first and can be produced by the smallest effective dosage involves the endoplasmic reticulum. The initial change is reversible, but, even more important, it is peculiar to rodents. There is no evidence that anything from the first increase in endoplasmic reticulum to the final development of a highly nodular liver with occasional displacement of cells to the lung has any bearing on the health of man or other animals in which the endoplasmic reticulum does not respond in this way.

7.1.10 Mutagenicity

DDT has been tested in a number of ways for possible mutational effects. Shirasu et al. (1976) listed DDT as a negative chemical in microbial mutagenicity screening studies on 166 pesticides. The test system consisted of rec-assay using H 17 Rec^+ and M 45 Rec^- strains of *Bacillus subtilis* and reversion assays without metabolic activation using auzotrophic strains of *Escherichia coli* (WP 2) and *Salmonella typhimurium* (Ames series). Further studies by the same authors, with metabolic activation, failed to reveal mutagenicity of DDT (Shirasu et al., 1977). McCann et al. (1975) and McCann & Ames (1976), reported negative results on DDE in *Salmonella typhimurium* testing with metabolic activation.

At a dosage of 105 mg/kg, DDT did not produce any increase in dominant lethals in mice (Epstein & Shafner, 1968). Concentrations of 10 mg/kg or higher produced chromosome breaks and exchange figures in a marsupial somatic cell line (Palmer et al., 1972). Saturated solutions produced chromosome breaks in the root tips of onion and other plants (Vaarama, 1947). A slight mutagenic effect in mammals has been reported by Markarian (1966). Deletions plus gaps were reported to be more common in the chromosomes of mice that had received DDT.

An unconventional test for mutagenicity involved examination of explants of pulmonary tissue from embryonic mice whose dams had been fed dietary concentrations of DDT of 10 and 50 mg/kg. An increase in diffuse hyperplasia and focal proliferation was observed, but a dosage-response relationship was not clear. Some of the embryos were allowed to live and the experiment was repeated in subsequent generations. There was no continuing progression of the reported changes in succeeding generations (Šabad et al., 1972).

7.1.11 Teratogenicity

When *p,p'*-DDT was administered to pregnant mice at a rate of 1 mg/kg on days 10, 12, and 17 of gestation, it was not teratogenic but did alter the gonads and decrease the fertility of the young, especially the females (McLachlan & Dixon, 1972). A single dose at the rate of 25 mg/kg or repeated doses of 2.5 (mg/kg) day given during pregnancy may be embryotoxic but not teratogenic to mice (Schmidt, 1973). The reason why one or a few doses during pregnancy may be embryotoxic although the same dosage is harmless, when administered during the entire reproductive period, is of theoretical but not practical importance.

Teratogenic effects of DDT have not been seen in studies of reproduction including those for 2 generations in rats, 6 generations in mice, and 3 generations in dogs (see section 7.1.7).

7.2 Acquisition of Tolerance to DDT

Because DDT stimulates microsomal, mixed-function enzymes and the action of these enzymes on DDT is one of detoxication, it might be expected that some tolerance might develop. Such tolerance has been demonstrated in the case of dieldrin; rats that received this compound for 28 days at a rate of 2 mg/kg survived 25 additional days at a rate of 5 mg/kg, a dosage that killed 70% of previously untreated rats (Hutterer et al., 1968). Apparently the possibility of tolerance to DDT has not been explored.

7.3 Factors Influencing DDT Toxicity

7.3.1 Dosage effect

7.3.1.1 *Dosage-effect of DDT*

Table 5 summarizes the acute oral and dermal toxicity of DDT in common laboratory animals, and Table 18 summarizes the subcutaneous intravenous and intraperitoneal toxicity. Both tables are condensed from an earlier review (Hayes, 1959) that gives references and additional details. It may be concluded that dissolved DDT is absorbed through all portals. Absorption of DDT powder through the skin is negligible. It is frequently impossible to put enough DDT dust on the skin of animals to kill them, so that an LD_{50} value for this formulation cannot be determined by the dermal route. Although formulation is important in determining the toxicity of

Table 18. Acute subcutaneous, intravenous, and intraperitoneal LD_{50} of DDT in common laboratory animals[a]

Species	Formulation	Subcutaneous (mg/kg)	Intravenous (mg/kg)	Intraperitoneal (mg/kg)
Rat	Water suspension or powder	>2000		
	Oil solution	200–1500	47	80–200
Mouse	Water suspension or powder	1000–1500		
	Oil solution	300		
Guineapig	Water suspension or powder			
	Oil solution	900		150
Rabbit	Water suspension or powder			
	Oil solution	250–>3200	30–41	<2100
Cat	Water suspension or powder			
	Oil solution	<650	32	
Dog	Water suspension or powder			
	Oil solution		68	
Monkey	Water suspension or powder			
	Oil solution		55	

[a] From: Hayes (1959).

DDT by other routes, the difference is not so great as it is in connexion with skin exposure. DDT is about 4 times more toxic when given intravenously than when given orally, and about 40 times more toxic when given intravenously than when given dermally.

In general, DDT appears to be more toxic as a solution in vegetable oil or animal fat than when given in some petroleum fraction. Petroleum may act as a laxative. The heavier fractions are never absorbed and DDT dissolved in such fractions has to be extracted from the solvent in order to show toxicity.

In summary, DDT is a compound of moderately acute toxicity. Compared with other organochlorine insecticides of equal or greater toxicity, it is remarkable in being only slightly absorbed by the skin.

The effects of repeated doses of DDT are summarized in Table 17.

The 90-dose oral LD_{50} of technical DDT in rats is 46.0 (mg/kg)/day (Gaines, 1969). The chronicity index is 5.4. Thus the compound has only a moderate tendency to cause cumulative effects, and this limited tendency is fully explained by the accumulation of DDT itself in tissues as a result of continuing intake. In fact, this accumulation, which is strictly dosage dependent, is detectable at all measurable levels of intake. The relationship in man is shown in Fig. 4 (p. 138).

If storage is considered undesirable *per se*, then DDT is without a no-injurious-effect level. However, the same may be said for all compounds that are absorbed, for the presence of all of them in the bodies of exposed organisms—perhaps at very low levels—may be assumed; failure to demonstrate low levels of storage does not depend on physiology but only on the limitations of analytical chemistry and on the lack of persistence of chemists.

A number of papers have reported no-effect levels for DDT within the variables investigated, namely: rat, 0.05 mg/kg (Lehman, 1965); dog, 8 mg/kg (Lehman, 1965); and monkey, 2.2–5.54 mg/kg (Durham et al., 1963).

There remain reports of effects in animals at the lowest dosages investigated. For example, decreased serum albumin and increased β- and γ-globulins in the blood of rats and rabbits maintained on a dosage of 0.2 (mg/kg)/day for 3–11 months was reported by Kagan et al. (1969).

In studies carried out in rats and dogs, toxicity, as measured by the maximum no-effect level, was seldom very different from the corresponding value resulting from 90 days of exposure at the same dosage range. The largest factor of difference observed when 33 chemicals were investigated in rats was 12 (20 for minimal effect level), and for half of them the factor was 2 or less. In 21, rat-to-dog comparisons of long-term toxicity, in no instance was the dog more sensitive than the rat (Weil & McCollister, 1963).

Although LD_{50} studies offer a poor basis for predicting long-term toxicity, the lowest dosage that will produce a minimum effect, when administered for the lifetime of rats, can be predicted with reasonable success from a test lasting only 7 days and with good success from a 90-day study (Weil et al., 1969). These results offer some perspective for judging the ultimate effect of exposure to compounds that have been commercially available for less than one human lifetime.

Summary of long-term toxicity studies. The lowest dosages that have been studied in animals are of the same order of magnitude as those encountered by men who make or formulate DDT and, therefore, hundreds of times greater than the dosages encountered by the general population. The animal studies have been continued long after a steady state of storage has been achieved. From the results it can be concluded that bioaccumulation sufficient to produce neurotoxicity or other clinical effects, including a reduction of the life span, can occur only at dosage levels substantially higher than those encountered by the most heavily exposed workers. DDT dosages encountered by workers produced a small but detectable increase in liver changes (hypertrophy, margination, and liposphere formation) in some groups of mice and rats. The same changes occurred in low incidence in control mice and rats but not in other animals (see section 7.1.9).

7.3.1.2 *Dosage-effect of metabolites and o,p'*-DDT

Acute oral LD_{50} values of DDT metabolites commonly found in tissues of excreta are shown in Table 19. Readily absorbable formulations of the metabolites are less toxic than the most absorbable preparations of the parent compounds (see for example Table 5).

Table 19. Oral LD_{50} values of metabolites of DDT

Compound	Species	LD_{50} (mg/kg)	Reference
DDE	rat, male	880	Gaines, 1960
DDE	rat, female	1240	Gaines, 1960
DDE	mouse	700	von Oettingen & Sharpless, 1946
DDE	mouse	1000	Domenjoz, 1946a,b
TDE(DDD)	rat, male	>4000	Gaines, 1969
DDA	rat	1900	Smith et al., 1946
DDA	rat, male	740	Gaines, 1960
DDA	rat, female	600	Gaines, 1960
DDA	mouse	720	von Oettingen & Sharpless, 1946
DDA	mouse	590	Domenjoz, 1946a,b

DDA. Rats tolerate higher tissue levels of DDA than of DDT. Eighteen hours after intravenous injection of DDA at a rate of 100 mg/kg, tissue levels were still higher than those usually found in animals, fatally poisoned by DDT (Judah, 1949).

DDA produces less injury to the liver than DDT but produces greater damage to the kidney especially at high intravenous dosages (Lillie et al., 1947). This is consistent with the finding of Spicer et al. (1947) that, following administration of DDT, DDA constitutes a higher proportion of DDT-related compounds in the kidney (25%) than in any other tissue, e.g., 12% in the liver, 10% in the brain, and even less in other tissues.

o,p'-DDT. At an oral dosage of 150 mg/kg, *p,p'*-DDT produces severe illness in all rats and kills about half of them, but *o,p'*-DDT at the same dosage does not produce illness even though the concentrations of the 2 compounds in the brain at various intervals after dosing are about the same. At a dosage of 3000 mg/kg, *o,p'*-DDT produces mild to moderate illness, and the concentration in the brain is 5–9 times the concentration of *p,p'*-DDT necessary to produce similar symptoms. Thus, *p,p'*-DDT appears to be inherently more toxic than the *o,p'*-isomer (Dale et al., 1966a).

7.3.2 Age and sex

Young animals eat more than adults in relationship to their body weight. For this and other reasons, they are often more susceptible than adults to poison in food. However, young animals are inherently less susceptible to certain compounds. There is no evidence that DDT is more toxic to the young than to the adults of any species, including man. In the rat, the young are less susceptible than adults to a single dose and about equally susceptible to repeated doses as shown in Table 20. According to Henderson & Woolley (1969), the relative insusceptibility of the young is associated with relatively poor absorption of DDT by the central nervous system and by the less inherent susceptibility of the young brain to DDT already absorbed by it. Further studies by the same authors (Henderson & Woolley, 1970) showed that fatal poisoning of both 10- and 60-day-old rats

Table 20. Effect of age on the toxicity of DDT to rats

Number of doses	Age	LD_{50} (mg/kg)[a]	Reference
1	newborn	4000	Lu et al., 1965
1	newborn	2356	Harrison, 1975
1	10 days	728	Henderson & Woolley, 1969
1	14–16 days	437.8	Lu et al., 1965
1	weanling	355.2	Lu et al., 1965
1	2 months	250	Henderson & Woolley, 1969
1	3–4 months	194.5	Lu et al., 1965
1	middle-aged	235.8	Lu et al., 1965
1	adult	225	Harrison, 1975
4	preweaning	279.2	Lu et al., 1965
4	adult	285.6	Lu et al., 1965

[a] Total intake one or more doses.

involved hyperexcitability and intense tremor followed by prostration and eventual respiratory failure. However, in the adult rat, DDT caused convulsions, an increase in respiration and heart rate, and a lethal increase in body temperature (40–42° C) prior to death, but the body temperature of the immature rat decreased during acute intoxication by DDT. The authors suggested that, whereas DDT is a direct depressant of respiration in both young and old rats, the additional toxic responses manifested by seizures and hyperthermia accounted for the increased lethality of DDT in mature animals.

There is virtually no sex difference in the acute toxicity of DDT to rats; the LD_{50} was 113 and 118 in males and females, respectively (Gaines, 1960). When DDT is fed to rats at ordinary dietary levels, the 2 sexes store it equally. However, at higher dosages, females store more of the compound; the difference is explained mainly by the lower activity of the liver microsomal enzymes in female rats and, only in part, by the relatively higher food intake of the females.

7.3.3 Nutrition

Fat. Nutrition influences the toxicity of DDT in connexion with both fat and protein. Fatness influences the amount that can be stored inactively in the body, and it is of importance in mitigating acute poisoning. This action of fatness or "good condition" has been noted in connexion with mammals (Spicer et al., 1947) and fish (Hoffmann & Surber, 1948). In contrast, laboratory animals are slightly more susceptible to repeated large doses administered as part of a diet containing a moderate proportion of fat (5% or more) than as part of a very low fat diet (0.5%) (Sauberlich & Baumann, 1947). This difference is thought to be associated with absorption from the gastrointestinal tract.

Rats that have stored large amounts of DDT in the fat may suffer tremors, if starvation or some other cause leads to a mobilization of their fat (Fitzhugh & Nelson, 1947). If DDT intake is stopped when starvation begins, and, if the concentration of DDT is measured only once following the interval of starvation, the results may be erratic, reflecting, to a greater or lesser degree, both mobilization of fat and excretion of metabolites as in studies reported by Deichmann et al. (1972).

However, in nature, starvation is more often partial than complete, and, if the original diet contains enough DDT to cause substantial storage, whatever food may be found in a period of scarcity is also likely to be contaminated. The initial effect of the mobilization of fat is to increase the concentration of DDT in the remaining fat and in other tissues. Excretion is increased in response to the increased tissue levels but may not be fast

enough to prevent the accumulation of a toxic concentration in the brain. If intake of DDT is stopped, the increased rate of excretion eventually leads to reduced storage (Dale et al., 1962). These findings in rats have been confirmed, in regard to both the initial increase in the concentration of DDT (Dedek & Schmidt, 1972; Štenberg & Diky, 1973) and the later reduction (Brodeur & Lambert, 1973). Similar findings have been reported in birds (Adamczyk, 1971).

The effect of fat mobilization on the toxicity of DDT is the same whether it is caused by withholding food or by disease that causes partial refusal of food (Hayes, 1975).

It is highly unlikely that poisoning by DDT will be precipitated in man by starvation because: (*a*) very few subjects store the compound in concentrations as high as those required to demonstrate the phenomenon in rats; and (*b*) the metabolic rate in man is so much slower than that in rats that elimination of DDT in man would counterbalance that produced by its mobilization.

Lipids. The association of lipids with the function of microsomal enzymes is generally recognized as is the fact that DDT induces these enzymes. Therefore, it might have been expected that DDT and essential fatty acids would interact. Tinsley & Lowry (1972) found that the growth of female rats receiving *p,p'*-DDT at a dietary level of 150 mg/kg was depressed, if they received a diet deficient in essential fatty acids, but was slightly stimulated, if they received the same diet supplemented with these acids. Another variable influenced by the same factors was the ratio of various liver lipids. The changes in fatty acid composition were related to the proliferation of hepatic smooth and endoplasmic reticulum; it was suggested that DDT influenced essential fatty acid metabolism by increasing the demand for them.

In contrast, a variety of diets (containing fats that may occur in the human diet and that were in approximately the same proportion as fats in the typical human food in the USA) had little or no influence on the storage of DDT and a wide range of pesticides fed to rats for 4 generations in a combination of rates 200 times those found in the Market Basket Study of food in the USA (Adams et al., 1974).

Ascorbic acid. In squirrel monkeys (and presumably in other species) only 2 days on an ascorbic acid-deficient diet impaired both the induction of *O*-demethylase and the stimulation of the glucuronic acid system by DDT (5 mg/monkey/day) (Chadwick et al., 1971a). In guineapigs, maintenance of induction of microsomal enzymes required a higher dietary level of ascorbic acid than prevention of scurvy (Wagstaff, 1971).

Protein. Smith & Stohlman (1945) found only slightly greater mortality and liver pathology in rats fed DDT at 500 mg/kg in a diet containing

protein at 80 g/kg than in one containing 280 g/kg. The finding that low dietary protein predisposes to DDT poisoning has been confirmed (Sauberlich & Baumann, 1947; Boyd & DeCastro, 1968, 1970; Boyd & Krijnen, 1969); however, even zero protein intake increased toxicity only 4-fold, the smallest factor observed in comparable studies of 9 pesticides. The effect of protein deficiency on toxicity may involve a crippling of the microsomal enzymes of the liver or it may act synergistically with compounds that cause anorexia. Rats fed a diet containing casein at 810 g/kg exhibited evidence of renal overload and were more susceptible to DDT (Boyd & DeCastro, 1970).

7.3.4 Species

The acute toxicity and metabolism of DDT were studied in mice and hamsters because of the marked difference in their susceptibility to liver tumours induced by DDT. The central nervous systems of the 2 species are equally sensitive, the concentration of DDT in their brains at death being similar. However, after an oral dosage of 500 mg/kg, the DDT concentration in the mouse brain was twice that in the hamster. This cannot be explained by a difference in absorption, metabolism, or excretion but apparently is due to a difference, in the permeability of the blood/brain barrier in the 2 species. When animals receive DDT at a dietary level of 250 mg/kg for 6 weeks, the residues in fat and liver were 7/8 times higher in the mouse, a fact only partially explained by the greater food intake of the mouse relative to body weight. Although urinary excretion of ^{14}C-DDT was similar in previously unexposed hamsters and mice, this excretion was stimulated in the hamster but little affected in the mouse by previous dietary exposure to DDT (Gingell & Wallcave, 1974).

Mice also differ from rats in the hormonal regulation of the basic activity of hepatic microsomal mixed-function enzymes as well as in the response of these enzymes to inducers (Chhabra & Fouts, 1974).

7.3.5 Other factors

A number of other factors are known to influence the toxicity of some compounds, and the degree of difference may be very great in isolated instances. Factors that have been reviewed elsewhere (Hayes, 1975) include (in addition to those listed above) interaction of compounds, strain, individual differences, isolation and crowding, other social and psychological factors, temperature, pressure and altitude, light and other radiation, circadian and other rhythms, seasonal differences, and relative humidity. None of these additional factors is known to have an important effect on the

survival of animals receiving DDT. The possibility of the interaction of DDT with aldrin, pyrethrin, piperonyl butoxide, malathion, dichlorophenoxyacetic acid (2,4-D), and a number of food additives has been explored systematically without finding anything but simple additive results (Fitzhugh, 1966). However, pyrethrins, especially synergized pyrethrins, have an additive and perhaps synergistic effect on the changes in liver morphology associated with repeated doses of DDT in rats (Kimbrough et al., 1968).

7.4 Human Studies

Oral exposure. Table 21 summarizes the effects of one or a few carefully measured oral doses of DDT. The results are consistent with those in accidents reported by Garrett (1947) and Hsieh (1954) in which it was possible to estimate accurately the amount ingested. It may be concluded

Table 21. Summary of the effects of one or a few oral doses of DDT on volunteers

Dose (mg) and formulation	Result	Reference
250 x 9, suspension	No effect.	Domenjoz, 1946a
1500, butter solution	No effect, but lice killed when fed 6 and 12 h after dose.	MacCormack, 1945
500, oil solution	No effect.	Neal et al., 1946
700, oil solution	No effect.	Neal et al., 1946
250, suspension	None except slight disturbance of sensitivity of mouth.	Velbinger, 1947a,b
250, oil solution	Variable hyperesthesia of mouth.	Velbinger, 1947a,b
500, oil solution	Variable hyperesthesia of mouth.	Velbinger, 1947a,b
750, oil solution	Disturbance of sensitivity of lower part of face; uncertain gait; peak reaction (6 h after ingestion) characterized by malaise, cold moist skin, and hypersensitivity to contact; reflexes normal.	Velbinger, 1947a,b
1000, oil solution	Same as above; no joint pains, fatigue, fear, or difficulty in seeing or hearing.	Velbinger, 1947a,b
1500, oil solution	Prickling of tongue and around mouth and nose beginning 2.5 h after dose; disturbance of equilibrium; dizziness; confusion; tremor of extremities; peak reaction (10 h after ingestion) characterized by severe malaise, headache, and fatigue; delayed vomiting; almost complete recovery in 24 h.	Velbinger, 1947a,b

that a single dose at the rate of 10 mg/kg produced illness in some but not all subjects even though no vomiting occurred. In general, smaller doses did not produce illness, although a dosage of 6 mg/kg produced perspiration, headache, and nausea in a man who was sickly and who was hungry at the time of eating. Persons who were made sick by 10 mg/kg did not have convulsions, but convulsions occurred frequently when the dosage level was 16 mg/kg or greater (Hsieh, 1954). Rarely, a dosage as high as 20 mg/kg might be taken without apparent effect (MacCormack, 1945). Dosages at least as high as 285 mg/kg have been taken without fatal result (Garrett, 1947). However, large doses lead to prompt vomiting, so that the amount actually retained cannot be determined accurately.

It has been noted, in the course of tests with volunteers, that dilute colloidal aqueous suspensions of DDT are odourless and tasteless (Domenjoz, 1946a; Hoffman & Lendle, 1948). Saturated alcoholic solutions of DDT have a weak aromatic taste or rather odour. Some people find these solutions slightly anaesthetic to the tongue (Hoffman & Lendle, 1948). The taste of DDT in vegetable oil is so slight that many persons could not identify capsules containing 0, 3.5, and 35 mg of DDT when they were presented separately but could arrange them in proper order when one of each was available for comparison (Hayes, personal communication, 1977).

The possible clinical effects of many repeated doses of DDT were first explored by Fennah (1945). Because of his interest in predicting the results of indiscriminate use, he expressed the exposures in terms of environmental levels rather than in dosage units. The exposures were clearly higher than those ordinarily encountered. In one test, lasting a total of 11.5 months, Fennah daily inhaled 100 mg of pure DDT and drank water dusted at the rate of 3240 mg/m^2. Much of the inhaled dust must have been deposited in the upper respiratory tract and swallowed. Later, for one month, Fennah ate food all of which had been sprayed at the rate of 2160 mg/m^2 after it had been served. No ill-effect of any kind was observed.

Some later studies on volunteers have been designed to explore the details of storage and excretion of DDT in man and to search for possible effects of dosages considered to be safe. In the first of these studies, men were given 0, 3.5, and 35 (mg/man)/day. These administered dosages, plus DDT measured in the men's food, resulted in dosage levels of 0.0021–0.0034, 0.038–0.063, and 0.36–0.61 (mg/kg)/day, respectively, the exact value depending on the weight of each individual. Six volunteers received the highest dosage of technical DDT for 12 months, and 3 received it for 18 months. A smaller number of men ingested the lower dosage of technical DDT or one of the dosages of *p,p'*-DDT for 12 or 18 months. No volunteer complained of any symptoms or showed, by the tests used, any sign of illness that did not have

an easily recognizable cause clearly unrelated to the exposure to DDT. At intervals, the men were given a systems review, physical examination, and a variety of laboratory tests. Particular attention was given to the neurological examination and liver function tests, because the major effects of DDT in animals involve the nervous system and the liver (Hayes et al., 1956). The same result was obtained in a second study in which the same dosages were given for 21 months and the volunteers were observed for a minimum of 27 additional months (Hayes et al., 1971). Information on storage and excretion gathered in these studies has already been discussed in sections 6.2.1.1 and 6.3.1.1.

Recently, DDT has been used on an experimental basis at dosage rates varying from 0.3 to 3 (mg/kg)/day for periods up to 7 months in an attempt to decrease serum bilirubin levels in selected patients with jaundice. No side-effects were observed. No improvement was noted in patients with jaundice based on cirrhosis who did not have any demonstrated liver enzymes deficiency. However, in a patient with familial, nonhaemolytic, unconjugated jaundice based on a deficienty of glucuronyl-transferase, treatment with DDT rapidly reduced the plasma bilirubin level to the normal range and relieved the patient of nausea and malaise from which he had suffered intermittently. The liver function tests as well as other laboratory findings remained normal. The improvement was maintained during the 6 months that DDT was administered, and had persisted for 7 additional months at the time the report was written. In this case, a dosage of 1.5 (mg/kg)/day produced a steady rise in plasma levels of *p,p'*-DDT from an initial level of 0.005 mg/litre to a maximum of 1.33 mg/litre at the end of treatment. At this time, the concentration in body fat was 203 mg/kg. Plasma levels fell slowly after dosing was stopped (Thompson et al., 1969). The highest daily intake in this series was 6 times greater than the highest level administered in earlier studies of volunteers and about 7500 times greater than the DDT intake of the general population. The highest value for *p,p'*-DDT in serum observed in the entire series was 1.330 mg/litre compared with 0.996 mg/litre, the highest value reported by Laws et al. (1967) for formulating plant workers.

Dermal exposure. Depending on dosage, oral administration of DDT to volunteers either did not produce any illness or produced only brief poisoning similar to that seen in experimental animals. The oral dosage necessary to produce any clinical effect was almost always 10 mg/kg or more. However, in 2 studies involving only 3 subjects in all, experimental dermal exposure to DDT was followed by fatigue, aching of the limbs, anxiety, or irritability, and other subjective complaints. Recovery was delayed for a month or more (Case, 1945; Wigglesworth, 1945). In neither study was there an independent control. Although the dosage was

unmeasured, the amounts of DDT absorbed must have been much smaller than those involved in the oral tests. One of the studies involved self-experimentation by one man. A similar but somewhat more severe test on 6 volunteers did not produce any toxic or irritant effects at all (Dangerfield, 1946). In view of all other experiments and extensive practical experience, it must be concluded that the illnesses reported by Wigglesworth and by Case were unrelated to DDT.

With the exceptions just mentioned, dermal exposure to DDT has not been associated with any illness or, usually, with any irritation (Wasicky & Unti, 1944; Draize et al., 1944; Cameron & Burgess, 1945; Fennah, 1945; Dangerfield, 1946; Chin & T'Ant, 1946; Domenjoz, 1946a; Haag et al., 1948). In fact, Hoffman & Lendle (1948) reported that even subcutaneous injection of colloidal suspensions of DDT in saline in concentrations up to 30 mg/litre did not cause irritation. Zein-el-Dine (1946) reported that DDT-impregnated clothing caused a slight, transient dermatitis, but the method of impregnation was not stated and the absence of solvent was not guaranteed. In other more thorough studies DDT-impregnated clothing was found to be non-irritating (Cameron & Burgess, 1945; Domenjoz, 1946a).

Small pads impregnated with different formulations of DDT were applied to the inner surface of the forearm of 32 volunteers whose cutaneous sensation had previously been measured for a period of 5 weeks. Pads impregnated with all the elements of the formulation except DDT were applied to the corresponding position of the other arm as a control. Powdered DDT and a solution of DDT at 50 g/litre showed little effect. Solutions in olive oil and petrolatum at 100 g/litre and 200 g/litre did not show any remarkable effect on sensation of pain, cold, or heat but reduced tactile sensation in most cases so that the minimum pressure that could arouse the tactile sensation was 1–2.5 g/cm^2 higher than for the control (Chim & T'Ant, 1946).

Respiratory exposure. Neal et al. (1944) reported almost continuous daily exposures to aerosols sufficient to leave a white deposit of DDT on the nasal vibrissae of the volunteers. This exposure produced moderate irritation of the nose, throat, and eyes. Except for this irritation during exposure, there were no symptoms, and laboratory tests and physical examination, including neurological evaluation, failed to reveal any significant changes. The studies by Fennah (1945), which involved both respiratory and oral exposure, did not produce any detectable ill-effects.

8. EFFECTS OF DDT ON MAN—EPIDEMIOLOGICAL AND CLINICAL STUDIES

8.1 Retrospective Studies on DDT-Exposed Populations

8.1.1 Epidemiological surveillance of persons occupationally exposed to DDT

The safety record of DDT is phenomenally good. It has been used for mass delousing in such a way that the bodies and inner clothing of thousands of people of all ages and states of health have been liberally dusted with the compound. By necessity, the persons applying the DDT work in a cloud of the material. Other subjects have sprayed the interior of hundreds of millions of homes in tropical and subtropical countries under conditions involving (Wolfe et al., 1959) extensive dermal and respiratory exposure. A smaller number of men have made or formulated DDT for many years. Extensive experience and numerous medical studies of groups of workers have been reviewed (Hayes, 1959). Dermatitis was commonly observed among men who used DDT solutions. The rashes were clearly due to the solvent, especially kerosene. As often happens with rashes caused by petroleum distillates, they were most severe in men when they first started work and cleared in a few days unless contamination was exceptionally severe. A smaller number of workers experienced mild narcotic effects (vertigo and nausea) from solvents when working in confined spaces. Gil & Miron (1949) reported that some persons suffered temporary irritability, fatigue, and other ill-defined symptoms after exposure to the dusty atmosphere of a delousing station, but the relation of these atypical findings to DDT was not clear. With these exceptions due largely to solvents, no illnesses clearly attributable to the formulations, much less to DDT, were revealed by the early studies.

Ortelee (1958) carried out clinical and laboratory examinations of 40 workers, all of whom were exposed to DDT and some of whom were exposed to a number of other pesticides. The men had been employed at this work with heavy exposure for 0.4 to 6.5 years and with slightly less exposure for as much as eight years. Exposure was so intense that, during working hours, many of the men were coated with a heavy layer of concentrated DDT dust. By comparing their excretion of DDA with that of volunteers given known doses of DDT, it was possible to estimate that the average dosages of 3 groups of the workers with different degrees of occupational exposure were 14, 30, and 42 mg/man per day, respectively. With the exception of the excretion of DDA and the occurrence of a few

cases of minor irritation of the skin and eyes, no correlation was found between any abnormality and exposure to the insecticide. Since very large doses of DDT injure the nervous system and liver of experimental animals, special attention was given to a complete neurological examination and to laboratory tests for liver function. Although a few abnormalities were revealed, none was detected in relation to DDT.

Thirty-five men employed from 11 to 19 years in a plant that had produced DDT continuously and exclusively since 1947 and, at the time of the study, was producing 2722 metric tonnes per month were studied by Laws et al. (1967). Findings from medical histories, physical examinations, routine clinical laboratory tests, and chest X-ray films did not reveal any ill-effects attributable to exposure to DDT. The overall range of storage of the sum of isomers and metabolites of DDT in the men's fat was 38–647 mg/kg compared with an average of 8 mg/kg for the general population. Based on their storage of DDT in fat and excretion of DDA in urine, it was estimated that the average daily intake of DDT by the 20 men with high occupational exposure was 17.5–18 mg/man per day compared with an average of 0.028 mg/man per day for members of the general population. There was significant correlation ($r = +0.64$) between the concentration of total DDT-related material in the fat and the serum of the workers. The average concentration in fat was 338 times higher than that in serum—a factor about 3 times greater than that for people without occupational exposure. Compared to members of the general population, the workers were found to store a smaller proportion of DDT-related material in the form of DDE; the difference was shown to be related chiefly to intensity rather than to duration of exposure. DDE is relatively a much less important and DDA a much more important excretory product in occupationally-exposed men compared with men in the general population. A further study of the same men involved in DDT production is discussed in section 8.2.5.

By far the largest number of heavily-exposed workers whose health has been investigated are those associated with malaria control in Brazil and India (WHO, 1973). In Brazil, periodic clinical examinations were made of 202 spraymen exposed to DDT for 6 or more years, 77 spraymen exposed for 13 years ending in 1959, and 406 controls. In the first examination carried out in 1971, minor differences between exposed and unexposed groups were observed in some neurological tests, but this result was not confirmed by the second examination in the same year nor in subsequent examinations. During a 3-year period, a survey of illnesses requiring medical care during the 6 months preceding each periodic medical examination failed to demonstrate any differences between exposed and control groups. A relatively small number of analyses indicated that the

variations present one year were lacking the next. The possibility of adaptive change (other than enzyme induction) has been suggested (Tocci et al., 1969), but this, like the reaility of the changes, remains unproved.

In some instances statistically significant differences have been found between workers and controls selected from the general population in connexion with parameters that have no known biochemical relationship to DDT and for which another explanation has not been excluded. For example, Keil et al., (1972) reported significant linear correlations between serum vitamin A and plasma DDT, TDE, and DDE levels.

There are a few reports of acute illness among workers attributed to exposure to mixtures of DDT and other materials. In so far as the dosage was very large, as in certain accidents that have occurred to individuals or groups in the general populations (see section 8.2.2), one would expect similar results. However, in at least one instance, headache, dizziness, nausea, vomiting, pain and numbness of the limbs, and general weakness beginning 1–1.5 h after entering a treated field (Kolyada & Mikhal'Chenkova, 1973) suggested food poisoning or hysteria.

Finally, there are studies of workers exposed to DDT and various other pesticides that are reported to have produced a variety of subjective and even objective medical findings. Interpretation of these reports is difficult because: (*a*) the findings do not resemble those of poisoned animals or of persons poisoned as a result of accident or suicide; and (*b*) the papers fail to report how the medical findings and the absenteeism of the pesticide workers compares with those of workers of comparable age, sex, and exertion who are not exposed to chemicals. The fact that the workers in question were exposed to mixtures of pesticides is not in itself an explanation because studies on many workers who were exposed to mixtures have not revealed any consistent differences between exposed subjects and unexposed controls. However, an explanation may lie in the degree of exposure. Reports of very high levels of organochlorine compounds in blood samples and of DDT in milk samples from populations in which illness was found are discussed in sections 6.2.1.2 and 6.3.1.2.

The reports under discussion tend to fall in 2 categories, those involving general debility and those involving a single organ or system. Conditions representative of general debility include dermatitis, subtle blood changes, general weakness, palpitations, functional angiospasm, headache, dizziness, diminished appetite, vomiting, lower abdominal pain, chronic gastritis, benign chronic hepatitis, isomnia, a sympathetic vascular/asthenic syndrome, vegetative dystonia, and confusion (Kostiuk & Mukhtrova, 1970; Bezugli et al., 1973).

Organs, systems, or functions that have been studied with the exclusion of other organs, systems, or functions of the same workers include: the

concentration of DDT in the blood of spraymen was about three times higher than that of controls.

In India, the blood levels of 144 spraymen were 7.5–15 times higher than those of the controls and were at least as high as those reported for workers who make and formulate DDT elsewhere (see Table 7). When the spraymen were examined, the only differences from the controls were that knee reflexes were brisker, slight tremor was more often present, and a timed Romberg test was more poorly performed by the spraymen. The positive results led to the selection of 20 men for re-examination by a neurologist who concluded that the differences found initially were not real or that the tests had returned to normal within the few months between the 2 examinations. The signs were not dosage-related, since they were not correlated with serum levels of DDT.

It has been known for several years that substantial doses of DDT and several other organochlorine insecticides stimulate the microsomal enzymes of the liver. This property of DDT was put to practical use in treating a patient with familial, nonhaemolytic, unconjugated jaundice, as described earlier. It was, therefore, entirely expected that persons with sufficient occupational exposure to a variety of pesticides would be able to metabolize a test drug (phenazone) more rapidly on the average than persons without occupational exposure were able to do. However, the change was not one of significantly increasing the fastest normal rate but of bringing all the workers up to a high level. There was no indication that the change had any effect on the workers' health (Laws et al., 1967, 1973; Kolmodin et al., 1969; Poland et al., 1970).

In addition to the studies already mentioned regarding workers with extensive storage, and excretion of DDT as a result of heavy exposure to DDT, studies have also been made of a larger number of workers with lesser storage and excretion following lesser exposure to DDT but greater exposure to other insecticides. Further studies (Long et al., 1969; Morgan & Roan, 1969, 1974; Warnick & Carter, 1972; Sandifer et al., 1972; Embry et al., 1972; Tsutsui et al., 1974; Ouw & Shandar, 1974) have failed to reveal effects of clinical significance among workers with prolonged, moderate exposure not only to organochlorine but also to organophosphorus and other types of insecticides. Small but statistically significant differences have appeared in the medical history or clinical laboratory results of some of these workers compared with the controls, but in no instance have the differences been of any medical importance, and dosage-response relationships have been unclear or absent. In several instances, the statistically significant differences have been opposite in different groups of workers; for example, creatinine phosphokinase activity was lower than that of controls in subjects applying the insecticide but higher in operators. Seasonal

respiratory system (Boiko & Krasniuk, 1969), liver (Bezuglyi & Kaskevich, 1969), stomach (Krasniuk & Platonova, 1969; Platonova, 1970), kidneys (Krasniuk et al., 1968), adrenals (Bakšeyev, 1973), skin (Karimov, 1969, 1970), and labour and the puerperium (Komarova, 1970; Nikitina, 1974). An indication that the difficulties under discussion are not serious is their reversal or prophylaxis by means of diet. Leščenko & Polonskaia (1969) described in detail two dietary supplements composed of ordinary foods plus sea-kale and a selection of vitamins and trace metals. Organochlorine-exposed workers who received these diet products showed a normalization of protein metabolism manifested by an increase in total serum protein, improved lipid metabolism, and enriched vitamin and trace element supplies in the organism. All of these effects led to an improvement in the detoxifying function of the liver, which was viewed as the most frequent site of adverse effects of exposure to organochlorine compounds.

8.1.2 Epidemiology of DDT poisoning in the general population: accidents and suicides

The only demonstrated effects of DDT on the general population are the storage of the compound and some of its derivatives in the tissues and their excretion in urine and milk. The facts were reviewed in sections 6.2.13 and 6.3.1.2. Briefly, DDT and some of its derivatives are found in all or nearly all persons in the population. The concentration is higher in tissues that have a high neutral fat content. Thus, for members of the general public the concentration of DDT-related compounds in adipose tissue is 100 or more times greater than the concentration in plasma (Laws et al., 1967). However, in spite of this great difference, sufficiently sensitive methods have demonstrated DDT in all tissues including the fetus and in all body fluids including human milk. These relationships are exactly what would be predicted from what is known of the storage of drugs and other compounds. Actual chemical demonstration of the distribution of DDT has been established for several years. Thus, its occurrence was first reported in human tissue (Howell, 1948), in tissue of the general population (Laug et al., 1951) in human milk (Laug et al., 1951), and in the human fetus (Denès, 1962).

There is extensive evidence that the mount of DDT and related material in the general diet in the USA has decreased as the use of DDT in that country has decreased, especially its use on forage. During the early 1950s, total DDT-related intake was approximately 0.265 mg/man per day and that for DDT was 0.163 mg/man per day (Walker et al., 1954). The average intake of DDT-related compounds based on a very large number of samples collected in different parts of the country during 1964–67 was 0.063

mg/man per day and that for DDT was 0.028 mg/man per day (Duggan, 1968). With decreased use of DDT, a gradual decrease in the storage of DDT and related material in human fat would be expected. Because only a few samples of fat were collected in the early studies of human tissue, there is some statistical uncertainty as to whether the decrease in storage that has been observed is real or whether it merely reflects variation due to sampling. In any event, by 1968, the average storage level of total DDT-equivalent material in fat was 7.67 mg/kg and that for DDT was 1.46 mg/kg. These averages were based on just over 3000 samples collected during the first half of 1968. The number of samples involved in this particular study was greater than the sum of all of the samples used in early studies. The best available values for concentrations in serum are 0.0294 mg/litre for total DDT-equivalent and 0.0047 mg/litre for *p,p'*-DDT.

Cases of accidental and suicidal poisoning in which the effects were clearly caused by DDT are summarized in Table 22. All of these cases involved ingestion. The signs and symptoms of poisoning were entirely consistent with those observed in volunteers, except that the spectrum of effects was broader because some of the accidental and suicidal doses were very high. A few persons have apparently been killed by uncomplicated DDT poisoning, but none of these cases was reported in detail. Death has been caused much more frequently by the ingestion of solutions of DDT, but in most instances the signs and symptoms were predominantly or exclusively those of poisoning by the solvent (Hayes, 1959). This does not mean that the toxicity of the solvent always predominates. For example, the recurrent convulsions in a case reported by Cunningham & Hill (1952), though more characteristic of poisoning by one of the cyclodienes, was certainly not typical of solvent poisoning. A 2-year-old child drank an unknown quantity of fly spray of which 5% was DDT, but the nature of the other active ingredients or the solvent was unknown. About 1 h after taking the material, the child became unconscious and had a generalized, sustained convulsion. Convulsions were present when the child was hospitalized 2 h after taking the poison, but the fits were controlled by barbiturates and other sedatives. Convulsions reoccurred on the fourth day and again on the twenty-first day but ceased each time following renewal of treatment. On the twelfth day, it was noted that the patient was deaf. Hearing began to improve about the twenty-fourth day and was normal, as were other neurological and psychic findings, when the patient was seen about 2.5 months after the accident.

Clinical effects of one toxicant may be modified by combining it with another. For example, prolonged illness would not be expected from ingestion of DDT at a rate of 27 mg/kg. However, when DDT and lindane were ingested in a suicidal attempt at dosages thought to be 27 mg/kg and

Table 22. Summary of the effects of the accidental or suicidal ingestion of DDT

Individual dose (mg) Formulation Number of persons	Results and reference
300–4500 in food 1 man	Onset in 1 h; vomiting; restlessness; headache; heart weak and slow; recovery next day (Mulhens, 1946).
unknown dose in tarts 25 men	Onset in 2–2.5 h; all subjects weak and giddy; 4 subjects vomited; 2 subjects hospitalized; one subject confused, uncoordinated, weak; one subject with palpitations and numbness of hands; recovery in 24–48 h (Mackerras & West, 1946).
5000–6000 in pancakes 3 men	Onset 2–3 h; throbbing headache; dizziness; incoordination; paraesthesia of extremities; urge to defaecate; wide nonreacting pupils; reduced vision; dysarthria; facial weakness; tremor; ataxic gait; reduced sensitivity to touch; reduced reflexes; positive Romberg; slightly low blood pressure and persistent irregular heart action; partial recovery in 2–3 days, but slight jaundice appeared 4–5 days after ingestion and lasted 3–4 days; all subjects normal 19 days after poisoning except for irregular heart action in one subject (Naevested, 1947).
2000 in pancakes 2 men	No illness (Naevested, 1947).
up to 20 000 in bread 28 men	Onset in 30–60 min in those most severely affected; men first seen 2–3 h after ingestion; in spite of severe early vomiting that reduced the effective dose, severity of illness and especially intensity of numbness and paralysis of extremities proportional to amount of DDT ingested; all but 8 men recovered in 48 h; 5 others fully recovered in 2 weeks, but 3 men still had some weakness and ataxia of the hands 5 weeks after ingestion (Garrett, 1947, 1950, unpublished data).
unknown dose in flour about 100 women	Onset about 3.5 h after ingestion; total of about 85 cases of which 37 were hospitalized; symptoms mild and similar to those in earlier outbreaks except for gastrointestinal disturbance in most severe cases including abdominal pain and diarrhoea as well as nausea; most subjects fully recovered in 24 h (Jude & Girard, 1949).
unknown dose 14 cases	Symptoms in established cases similar to those reported earlier (Francone et al., 1952).
286–1716 in meatballs 8 cases, 11 exposed	With the exception of one man who was already sick when he received a dosage of 6 mg/kg, poisoning did not occur at dosages of 5.1–10.3 mg/kg. Ingestion of 16.3–120.5 mg/kg produced excessive perspiration, nausea, vomiting, convulsions, headache, increased salivation, tremors, tachycardia, and cyanosis of the lips. Onset varied from 2–6 h depending on dosage. Recovery required as much as 2 days (Hsieh, 1954).
unknown dose 1 case	Death 13 h after suicidal ingestion (Committee on Pesticides, 1951).
unknown dose 22 unrelated cases	Twenty-two separate cases, including 15 attempted suicides; some complicated by solvents; 3 deaths (Committee on Pesticides, 1951).

18 mg/kg, respectively, clinical remission of convulsions and liver involvement was delayed until the twentieth day, and the EEG did not return to normal until the thirty-ninth day (Eskenasy, 1972).

There have not been any accidents or suicides involving respiratory or

dermal exposure to DDT leading to recognized signs and symptoms of poisoning, even though sufficient respiratory exposure to aerosols or sufficient dermal exposure to solutions can cause poisoning in animals; the difference is certainly one of dosage.

It has been alleged that DDT causes or contributes to a wide variety of diseases of man and animals not previously recognized as being associated with any chemical. Such diseases include cardiovascular disease, cancer, atypical pneumonia, retrolental fibroplasia, poliomyelitis, hepatitis, and "neuropsychiatric manifestations" (Biskind & Beiber, 1949; Biskind, 1952, 1953; and others). Without exception the causes of these diseases were unknown or at least unproved at the time of the allegation. Needless to say, the charge that DDT predisposes to poliomyelitis was dropped after the disease was controlled through the use of vaccines. Unfortunately, there is no immediate possibility of controlling cardiovascular disease, cancer, or many of the less common conditions in man that have been ascribed to DDT. In the meantime, such irresponsible claims could produce great harm and, if taken seriously, even interfere with the scientific search for true causes and realistic means of preventing the conditions in question.

8.1.3 Epidemiology of DDT poisoning in infants and young children

Nothing is known fundamentally to distinguish the epidemiology of DDT poisoning among children from that among adults. In both instances, poisoning has never been confirmed, except where the dosage was large, usually as the result of an accident and usually involving gross carelessness. Probably a larger number of cases have occurred in infants simply because they are more likely to eat and drink formulations that they find in unlabelled containers frequently originally intended for food. However, as far as DDT is concerned, all the large outbreaks of poisoning have involved adults under military conditions, thus children were not exposed. As might be expected, some deaths of adults but none of children has apparently involved suicide. Most, if not all, cases in adults were uncomplicated poisoning by DDT, but several cases in children involved the drinking of solutions so that the signs and symptoms were actually caused by the solvent (Reingold & Lasky, 1947).

8.2 Clinical and Epidemiological Studies of the Effects of DDT on Specific Organs and Systems

8.2.1 Haemopoietic system and immunology

In acute poisoning, a slight decrease in haemaglobin and a moderate leukocytosis without any constant deviation in the differential white count

have been observed in volunteers (Velbinger, 1947a,b). These findings are considered secondary to the neurological effects.

There is a strong tendency to blame blood dyscrasias, other manifestations of "hypersensitivity", and, in fact, many diseases of unknown cause on any new chemical that gains widespread attention. DDT was no exception. A review of the early literature (Hayes, 1959) indicates that blood dyscrasias and an unbelievable range of other diseases were, in fact, blamed on DDT. Only a circumstantial relationship was ever established between these diseases and exposure to DDT, and this is true of the small number of reports of blood dyscrasias (Murray et al., 1973) or angioneurotic oedema (Vanat & Vanat, 1971) that have appeared recently. Later, fewer new reports appeared linking DDT to diseases of unknown cause, although the use of DDT increased greatly. It is true that available tests do not make it possible to exclude a particular compound as a cause of an isolated case of blood dyscrasia. However, it is noteworthy that the rate at which these disorders occur has remained essentially unchanged since before DDT was introduced (Hayes, 1975).

8.2.2 Nervous system

The effects of carefully measured doses of DDT that proved to be just above the minimum toxic level are best described from studies of volunteers (see section 7.4). Similar early signs and symptoms have been encountered in cases of accidental poisoning that frequently progressed to more severe illness as described in section 8.1.2.

Briefly, the earliest symptom of poisoning by DDT is hyperaesthesia of the mouth and lower part of the face. This is followed by paraesthesia of the same area and of the tongue and then by dizziness, an objective disturbance of equilibrium, paraesthesia and tremor of the extremities, confusion, malaise, headache, fatigue, and delayed vomiting. The vomiting is probably of central origin and not due to local irritation. Convulsions occur only in severe poisoning.

Onset may be as soon as 30 min after ingestion of a large dose or as late as 6 h after smaller but still toxic doses. Recovery from mild poisoning is essentially complete in 24 h, but recovery from severe poisoning requires several days. In two instances, there was some residual weakness and ataxia of the hands, 5 weeks after ingestion.

Electroencephalograms were obtained from 73 workers exposed to DDT, HCH, and chlorobenzilate for periods ranging from 7 months to 20 years. Just over 78% of the records were normal and 21.9% were abnormal. The most severe changes involved persons exposed to the 3 compounds for 1–2 years; less severe changes were seen with either shorter or longer exposure.

The changes were not correlated with age, the range and mean of age for those judged abnormal being almost identical to these values for persons considered normal. Some of the records showed bitemporal sharp waves with shifting lateralization combined with low voltage theta activity. Other records showed spike complexes, paroxymal discharges composed of slow and sharp waves most pronounced anteriorly, and low voltage rhythmic spikes posteriorly. None of the persons examined showed any abnormal clinical neurological finding (Israeli & Mayersdorf, 1973; Mayersdorf & Israeli, 1974). The incidence of abnormal electroencephalograms in the general population is 9.0% or 9.2%, according to other investigators cited by Israeli & Mayersdorf. Czegledi-Janko & Avar (1970) considered that nonspecific EEG abnormalities occurred in 10–20% of the general population.

The frequency and degree of olfactory disorders, especially in the ability to detect peppermint and acetic acid in an olfactory analyser, were reported to be greater among persons exposed to pesticides, and to increase with duration of exposure (Salihodžaev & Ferštat, 1972). Whether any of the persons exposed to pesticides experienced any clinical difficulty or social inconvenience associated with olfactory sensation is not clear.

8.2.3 Renal system

There is no indication of renal damage in people, accidentally poisoned by DDT, or in workers heavily exposed to it.

8.2.4 Gastrointestinal system

Except for vomiting, which probably is of central origin, the gastrointestinal system has not been affected in acute poisoning.

8.2.5 Liver

Involvement of the liver has been mentioned in only a small proportion of cases of accidental poisoning by DDT. In 3 men who ate pancakes made with DDT and thus ingested 5000–6000 mg each, slight jaundice appeared after 4–5 days and lasted 3–4 days (Naevested, 1947). Hepatic involvement and convulsions were reported in an unsuccessful suicide attempt by ingesting DDT and lindane (Eskenasy, 1972).

Laws et al. (1973) made a detailed study of the liver function of 31 men who had made and fomulated DDT and who had been the subjects of an earlier study (see section 8.1.1). Judging from their excretion and storage, the men's exposure was equivalent to oral intakes of DDT at rates ranging

from 3.6 to 18 mg/man per day for periods ranging from 16 to 25 years and averaging 21 years. All tests were in the normal range for total protein, albumin, total bilirubin, thymol turbidity, and retention of sulfobromophthalein sodium (BSP). One man had mild elevations in levels of both alkaline phosphatase (EC 3.1.3.1) (16 units) and serum glutamic pyruvic transaminase (EC 2.6.1.2) SGPT (42 units). Another man had an alkaline phosphatase concentration of 14 units, while a third man had an SGPT level of 49 units. The α-fetoprotein test was negative for all 20 of the men tested.

8.2.5.1 *Liver enzymes*

The induction of human microsomal enzymes of the liver by various drugs was well known when Kolmodin et al. (1969) demonstrated this effect in workers exposed to a variety of pesticides, including DDT. Later, Poland et al. (1970) showed that workers who made and formulated DDT and absorbed it at an average rate of about 0.25 (mg/kg)/day metabolized phenylbutazone more rapidly on the average than controls and excreted more 6β-hydroxycortisol. Occupational exposure increased the drug-metabolizing ability of some workers, so that they all metabolized test drugs with the efficiency of those members of the general population who were most efficient in this respect. The concentration of *p,p'*-DDT in the serum of the workers studied by Poland averaged 0.573 mg/litre. In other workers with less exposure to DDT, as indicated by average serum levels of 0.052 mg/litre, there was no increase in the urinary excretion of D-glucaric acid, which is increased by a number of exogenous and endogenous substances that induce microsomal enzymes (Morgan & Roan, 1974).

Thompson et al. (1969) demonstrated, in a different way, the induction of microsomal enzymes by using DDT at a dosage of 1.5 (mg/kg)/day for 6 months in the successful treatment of unconjugated hyperbilirubinaemia. In a similar way, Rappolt (1970) used DDT to promote metabolism of an overdose of phenobarbital. It is of interest that the levels of DDE in the serum of some workers studied by Morgan & Roan (1974) approached those of workers studied by Poland et al. (1970). The lack of induction in one group and its presence in the other suggests that enzymes are induced in man more readily by DDT than by DDE.

DDT promotes its own metabolism in some species of laboratory animals. That the same is true in man is indicated by the fact that storage of DDT is relatively less at higher dosages (see Fig. 4). However, the metabolism and subsequent excretion of DDT can be promoted even more by other inducing agents. Patients who received phenobarbital or, more especially, phenytoin stored much less DDT than other persons with similar exposure to DDT (Davies et al., 1969a; Edmundson et al., 1970b; Watson et al., 1972). This result concerning phenytoin was confirmed by McQueen

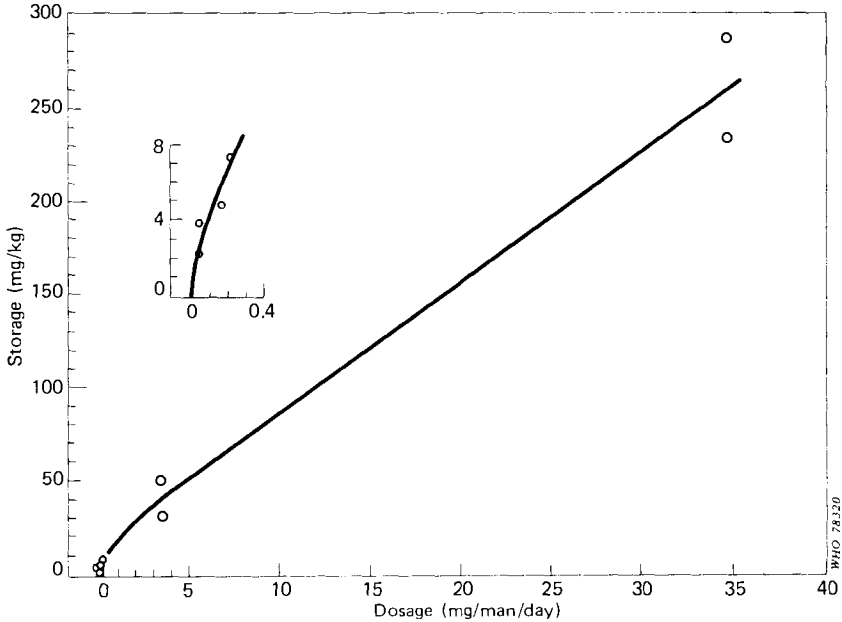


Fig. 4. Concentrations of DDT in body fat plotted against daily dosages (From: WHO, 1973).

et al. (1972) who also showed that other drugs produced a smaller but still highly significant reduction in DDT storage. Establishment of a reduced equilibrium appeared to require about 2 months. Within this period, the regression of the level of DDT plus DDE on duration of treatment with phenytoin was highly significant ($P < 0.001$).

At the end of 9 months' treatment, the body fat of nonepileptic volunteers given phenytoin at a rate of 300 mg/man per day contained an average of 25% of the DDT and 39% of the DDE concentrations originally present before administration of the drug (Davies et al., 1971).

The same was true of workers whose exposure was greater than that of the general population. Maintenance doses of phenobarbital, phenytoin, or a combination of the two kept the storage levels of several organochlorine insecticides in epileptic workers as low as, or lower than levels in the general population (Schoor, 1970; Kwalick, 1971).

8.2.5.2 *Other biochemical observations*

A positive linear correlation has been reported for the concentrations of vitamin A and of DDT-related compounds in the serum of men with at least 5 years of occupational exposure to DDT. However, the workers' DDT levels were little higher than those of persons in the general population (see

Table 7), and their vitamin A levels were within normal limits (Keil et al., 1972). Perhaps they were better fed than the controls.

Compared to 86 unexposed workers, the serum total cholesterol values of 206 workers in a chemical plant where unidentified organochlorine insecticides were made and formulated were higher in workers who were less than 25 years old, lower in those between 25–34 years and 35–44 years, and higher in those who were 45 years old or more. The differences were significant only for the oldest groups (Wassermann et al., 1970a).

8.2.6 Cardiovascular system

The small amount of knowledge concerning the effect of DDT on the human heart fails to show whether cardiac arrhythmia might be a possible cause of death in acute poisoning, as is true in some species of laboratory animals. Palpitations, tachycardia, and irregular heart action have been noted in some, but not all cases of acute poisoning (Mackerras & West, 1946; Naevested, 1947; Hsieh, 1954).

8.2.7 Reproduction

There is no indication that DDT has influenced reproduction except to increase it as an indirect result of disease control, especially malaria control.

After Laws et al. (1967) had completed their study, Wilcox (1967) found that the 36 most heavily exposed workers involved had fathered 58 children before they began working at the DDT factory and 93 children afterwards.

O'Leary et al. (1970c) did not find any significant relationship between abortion and blood levels of DDT-related compounds.

8.2.8 Endocrine organs

Average protein-bound iodine (PBI) levels of 0.0542 and 0.0693 mg/litre, respectively, were reported in the sera of 42 workers occupationally-exposed to organochlorine insecticides and in 51 workers who were not exposed. The difference was statistically significant even though all values fell within the normal range of 0.04–0.08 mg/litre (Wassermann, D. et al., 1971). It was not recorded whether the workers involved were from the same factory as those with 10 or more years of occupational exposure whose plasma DDT levels were reported by Wassermann et al. (1970c) (see Table 7). The small difference in PBI levels is difficult to evaluate. It was the view of Clifford & Weil (1972) that there was not any evidence that occupational exposure had had an effect on human endocrine organs.

TDE. Following the demonstration (discussed in section 7.1.8) that TDE

caused atrophy of a part of the adrenal cortex of dogs, *o,p'*-TDE, and to a lesser degree *m,p'*-TDE, have been used in man, under the name of mitotane, in the hope of controlling excessive cortical secretion or of reducing the size of adrenal tumors. The underlying condition may be hyperplasia or adrenocortical carcinoma. The dosage given has varied from 7 to 285 (mg/kg)/day, but a dosage of approximately 100 (mg/kg)/day for many weeks has been necessary to produce any benefit in man (Bergenstal et al., 1960; Wallace et al., 1961; Gallagher et al., 1962; Verdon et al., 1962; Bledsoe et al., 1964; and Southern et al., 1966a,b).

The effects of idiopathic hyperplasia may be controlled; in fact a state of adrenal insufficiency may be produced (Canlorbe et al., 1971; Sizonenko et al., 1974).

o,p'-TDE may also give symptomatic relief of excessive adrenocortical activity secondary to a tumour that produces ACTH (Carey et al., 1973).

A favourable response was produced in about one-fourth to one-half of patients with inoperable adrenocortical carcinoma (Canlorbe et al., 1971; Hoffman & Mattox, 1972; Lubitz et al., 1973; Montgomery & Struck, 1973). In fact, an occasional cure, involving complete regression of metastases, was produced by chemotherapy including *o,p'*-TDE (Perevodchikova et al., 1972; Schick, 1973). More commonly, symptoms were relieved and life was prolonged by little more than 7–8 months (Canlorbe et al., 1971; Hoffman & Mattox, 1972; Lubitz et al., 1973). The success of treatment was often indicated early on by a reduction in steroid excretion (Hoffman & Mattox, 1972; Lubitz et al., 1973).

The large dosage of *o,p'*-TDE necessary to produce clinical benefit often produced general lassitude, anorexia, nausea, vomiting, diarrhoea, and dermatitis (Naruse et al., 1970; Hoffman & Mattox, 1972; Nitshke & Link, 1972; Perevodčikova et al., 1972; Lubitz et al., 1973). Apathy ranged from mild dulling of interest to profound psychotic depression (Hoffman & Mattox, 1972). More rarely, gynaecomastia, haematuria, leukopenia, and thrombocytopenia have been reported (Luton et al., 1972; Perevodčikova et al., 1972). The symptoms disappeared soon after administration of the drug ceased or when the dosage was reduced (Perevodčikova et al., 1972).

Even large, therapeutic doses of *o,p'*-TDE did not cause histological alterations in the adrenals in man (Wallace et al., 1961). Furthermore, dosages in the therapeutic range (specifically those between 110 and 140 (mg/kg)/day did not produce any detectable injury to the liver, kidney, or bone marrow. All patients treated in this way experienced significant anorexia and nausea, and some showed central nervous system depression varying from lethargy to somnolence. These toxic effects cleared when dosing was discontinued (Bergenstal et al., 1960).

Kupfer (1967) reviewed extensive literature that indicated that the

effect in man and other species, except the dog, is caused by stimulation of corticoid metabolism by massive doses of *o,p'*-TDE and not by any direct effect on the adrenal. Southern et al. (1966a,b) agreed that the effect was predominantly extra-adrenal in man, when the drug was first given, but offered evidence that adrenal secretion of cortisol was eventually reduced. However, even if therapeutic doses eventually have a direct effect on the adrenal, doses encountered by workers exposed to technical DDT do not (Clifford & Weil, 1972; Morgan & Roan, 1973).

8.2.9 Carcinogenicity

Laws et al. (1967) did not find any case of cancer or blood dyscrasia among the 35 heavily exposed workers in a DDT factory nor did the medical records of 63 men who had worked there for more than 5 years reveal these diseases. Two men were employed who had a history of successfully treated cancer before they came to work, but no employee had contracted cancer during the 19 years that the plant had been in operation; during this period, the work force varied from 111 to 135.

In the USA, the total death rates for cancer of the liver and its biliary passages (classified individually as "primary", "secondary", and "not stated whether primary or secondary") lead to the conclusion that there has been a significant, almost constant decrease in the total rate of liver cancer deaths from 8.8 in 1930 to 8.4 in 1944 (when DDT was introduced) to 5.6 in 1972. This almost constant decline in total liver cancer death rates for the past 42 years offers no evidence of any increase in liver cancer deaths since the introduction of the first organochlorine pesticide into the environment. The decrease in liver cancer deaths is even more significant in light of the increasing life span of the general population in the USA, which has resulted in an increased percentage of the population at risk from cancer over these years. In spite of the limitations inherent in the interpretation of such data, this record is a reminder that, more than 30 years after the introduction of DDT, there is no evidence, whatsoever, that DDT is carcinogenic in man (Deichmann & MacDonald, 1976, 1977).

In the USA, the incidence of cancer is lower in rural counties than in metropolitan areas in general (Mason et al., 1975).

It is sometimes implied that epidemiological evidence is useless for revealing the carcinogenicity of a material for man unless it involves large numbers of people who have been exposed to the material for most or all of a lifetime. The fact is that some human carcinogens have been detected through their occurrence in high incidence in small groups for periods of much less than 25 years. What was commonly considered the first recognition of chemical carcinogenesis in man depended on the observations

made by a single surgeon (Pott, 1775) in a small fraction of his patients. Such was the intensity of the exposure of the apprentices of chimney sweepers that cancer of the scrotum often appeared at puberty. The editor responsible for compiling the writings of Pott (1790) added a footnote indicating that he had seen such a cancer in "an infant under eight years of age". It must be understood that boys did not usually become apprentice chimney sweepers before they were four-years-old. In connection with tumours of the bladder mainly caused by β-naphthylamine but to a lesser degree by other aromatic amines, Hueper (1942) reviewed cases in which the time from the first exposure to recognition of symptoms was 8–41, 9–28, and 2–35 years; in one series of 83 cases, 71% of the tumours appeared from 1 to 15 years after exposure. The same author cited reports (p. 104) of cases of malignant epitheliomas in persons exposed to pitch for 18, 24, 24, and 36 months, respectively. Kleinfeld (1967) reported 50–76% incidence of bladder cancer among several groups of workers. He also noted a sharp drop in incidence of this condition following decrease—but not discontinuation—of occupational exposure to β-naphthylamine.

8.2.10 Mutagenicity

Evidence regarding the mutagenic activity of DDT and its significance in man is uncertain partly because the chromosomal changes that are examined are sensitive to viral infections and chemotherapy. The latter may not be recognized at the time of sampling and may not have been shown to injure health through a mutagenic mechanism.

Comparing samples collected in winter and during the peak season of pesticide application, a slight increase in chromatid breaks was reported in the cultured lymphocytes of workers exposed to a wide variety of insecticides said to include DDT, although this was claimed at a time when the use of DDT was banned. A somewhat larger increase was reported for men exposed mainly to herbicides (Yoder et al., 1973). In another study, lymphocytes cultured from workers with an average DDT plasma level of 0.999 mg/litre showed significantly more chromosomal and chromatid aberrations than cells cultured from controls with an average plasma level of 0.275 mg/litre. The difference was not significant in other comparisons in which the average plasma levels were 1.030 versus 0.380 mg/litre and 0.240 versus 0.030 mg/litre, respectively (Rabello et al., 1975). Examination of all of the data presented by the authors suggests a simple dosage-effect relationship was present, with a detectable effect starting somewhere between 0.2 and 0.4 mg/litre and increasing at levels higher than 0.4 mg/litre.

8.3 Factors Influencing DDT Toxicity

There is no evidence that any factor except dosage is of practical importance in determining DDT toxicity in man. Factors that have been considered as possibly affecting asymptomatic storage of DDT include age, sex, and race. Differences observed in connexion with these factors are small, medically insignificant, and probably secondary to dosage (Hayes, 1975).

Storage has also been reported to be greater in the tissues of people with certain diseases (Deichmann & Radomski, 1968; Radomski et al., 1968; Vas'kovskaja, 1969; Dacre & Jennings, 1970; Jonczyk et al., 1974). Again, the reported differences are small, and the highest values for the samples in question are small compared with those found in healthy workers (Hayes, 1975). Furthermore, a number of authors have reported a similar range of storage in persons undergoing minor, elective surgery and in those who have died from various causes (Hayes et al., 1958; Dale et al., 1965; Robinson et al., 1965; Wassermann et al., 1965). Some authors (Hunter et al., 1963; Robinson et al., 1965; Hoffman et al., 1967; Hoffman, 1968; Morgan & Roan, 1970) have failed to find any relationship between storage of insecticides and the cause of death. Where a relationship was found, there was often the possibility that the higher values were found in diseases that involved some degree of wasting prior to death. Casarett et al. (1968) found that higher values occurred in persons who had 3 characteristics in common: emaciation, cancer, and widespread abnormality of the liver.

A slightly greater storage of DDT and DDE that was reported in persons who underwent splenectomy for hepatosplenic schistosomiasis compared with those operated on for other conditions, mainly hernia was statistically significant. No such difference was observed in connexion with dieldrin, β-HCH, or heptachlor epoxide (Wassermann et al., 1975). Whereas it was speculated that the increased storage of DDT and DDE might have been the result of a reduction in metabolism, secondary to liver injury, the possibility of greater exposure as a result of greater use of DDT in irrigated areas was not excluded.

8.4 Treatment of Poisoning in Man

No useful guidance regarding treatment has been obtained from the very few cases of DDT poisoning that have occurred. Animal studies indicate that sedatives, ionic calcium, and glucose or another ready source of energy would be useful. On the basis of experience in treating people poisoned by different convulsive poisons, it seems likely that diazepam would be beneficial.

9. EVALUATION OF HEALTH RISKS TO MAN FROM EXPOSURE TO DDT AND RELATED COMPOUNDS

9.1 Relative Contributions of Food, Water, Air, and Miscellaneous Sources to Total Intake

9.1.1 Adult members of the general population

Food represents the major source of intake of DDT in the general population. It has been estimated (section 5.1.5) that over 90% of the DDT stored in the general population is derived from food. Around 1965, when the use of DDT was at its peak, intake in the USA was approximately 0.04 mg/man per day from food, less than 0.000046 mg/man per day from water, less than 0.00006 mg/man per day from urban air and less than 0.0005 mg/man per day from the air in small agricultural communities. The reason for the qualification "less than", is that the intakes were calculated from the highest concentrations reported in drinking-water and air.

Although total intake of DDT from food has not been measured in some parts of the world, worldwide measurements of the storage of DDT and its metabolites in human body fat indicate that the extremes of total exposure have varied by a factor of about 10, but that total exposure for most populations has varied by a factor of no more than 3 (see Table 9).

DDT in the dust in a house (as indicated either by a history of extensive household application of insecticides or by the finding of relatively high levels of DDT in house dust) can contribute to the storage of DDT-related compounds in persons living in the house (section 5.1.4). However, although the contribution of house dust to DDT intake has been established, it is not quite clear how this contribution occurs. Some of the dust may contaminate food in the process of preparation and some may be inhaled and later swallowed after deposition in the upper airway. It is difficult to believe that enough DDT is present in such houses in the form of vapour or respirable dust to cause a substantial increase in the total exposure of the inhabitants, but no critical study has been made of this matter. Clearly, some DDT will enter by the respiratory route.

9.1.2 Infants and children

At birth, infants tend to have slightly lower levels of DDT than adults in the same population. This is because the placenta offers partial protection against the passage of DDT and related compounds.

Although human milk tends to have a somewhat higher concentration of DDT than cow's milk (see section 6.3.1.3), the difference, if any, that this makes to the rate at which breast-fed and bottle-fed babies store the compound has not been established. It is possible that the conditional acceptable daily intake (ADI) might be exceeded in an infant wholly fed on breast milk. However, the ADI is calculated on the basis of lifetime exposure, and short-term variations can be regarded as not having any significance.

The only really important way in which the exposure of infants and children differs from that of adults in the same community involves accidental exposure (see section 8.1.3).

9.1.3 Occupational groups

Occupational exposure (section 5.3) to DDT is initially almost exclusively through the respiratory and dermal routes. However, the particles of many insecticidal dusts, wettable powders, and sprays are too large to reach the lower respiratory tract. As a result, most of the particles inhaled are deposited in the upper respiratory tract, carried to the pharynx by ciliary action, and eventually swallowed (section 6.1.1).

Although dermal exposure to DDT is high under some occupational situations, the effect is minimal because the compound is so poorly absorbed through the skin (section 6.1.3). The excellent safety record of DDT, never matched by any other insecticide used in antimalaria campaigns, other vector control programmes, and agriculture, is based mainly on its poor absorption through the skin.

The number of people with full-time occupational exposure to DDT alone is small. For example, at the time of one study, the only factory making the compound in the USA produced 2722 metric tonnes per month using a work force of about 145. Following the recommendations of an Expert Committee, the World Health Organization studied spraymen who had applied only DDT for 5 years or more. Only 272 suitable subjects could be located in Brazil and only 144 in India. The concentrations of DDT and its derivatives in the blood of the preliminary and main study groups in India were 0.761 and 1.272 mg/litre, respectively. The blood levels of spraymen in Brazil were about 3 times those of the controls.

The absorbed dosage of the men who made and formulated DDT for 10 years or more was about 18 mg/man per day (see section 8.1.1). The exposure of other workers, notably those applying DDT for agricultural purposes has usually been an order of magnitude less (see Table 7).

9.2 Effects of Exposure

No adverse effects have been described at repeated dosages of 1.5 (mg/kg)/day or less (see section 7.4). The large number of measurements that have been made on samples from human populations have not made it possible to define a maximum dosage that man can absorb without any adverse effect but have highlighted the finding that the high levels found in volunteers and workers were harmless for at least 25 years.

Table 23 summarizes the clinical aspects of DDT in man.

Table 23. Dosage-effects of DDT in man

Single dose (mg/kg)	Observation
Unknown	Fatal
16–286	Prompt vomiting at higher doses (all poisoned, convulsions in some)
6–10	Moderate poisoning
Repeated exposure (mg/kg)/day	
1.5	Administered as therapy for 6 months[a]
0.5	Administered to volunteers for 21 months[a]
0.5	Exposure of workers for 6.5 years[a]
0.25	Exposure of workers for 25 years[a]
0.0025	Intake of population in the USA, 1953–54[a]
0.0002	Intake of population in the USA, 1969–70[a]

[a] Without any adverse effect.

In considering the safety of workers who are employed in the DDT industry, it is useful to consider the results of animal experiments. Rats withstand a daily dosage at least 10 times that of these workers without any detectable clinical effect (section 7, Table 17), although minimal reversible tissue changes may be present. Dogs and monkeys also withstand a daily dosage 10 times higher than that of the workers, but they do not show the tissue changes, which seem to be peculiar to some rodents. Because workers have tolerated high dosages of DDT for over a fifth of a lifetime without detectable harm and since animals withstand larger dosages for an entire lifetime without injury, there is good reason to predict the continuing safety of the workers.

The experience already gained from workers can be used to predict the future safety of the general population in relation to DDT. Many workers have now been exposed to DDT for much more than one-fifth of their life span. Since they have not suffered detectable harm, it seems most unlikely that the general population will be harmed by dosages 200–1250 times smaller than those to which the workers are exposed. It has been shown for at least 2 animal species that toxicity resulting from a lifetime of exposure is

seldom very different from toxicity resulting from 90 days of exposure at the same dosage rate. The largest factor of difference observed when 33 chemicals were investigated was 20, and, for half of them, the factor was 2 or less (see section 7.3.1.1). Ninety days constitute about one-eighth of the lifespan of a rat, and this is less than the fraction of the human life span that has been studied so far.

9.3 Carcinogenicity and Mutagenicity

Liver tumours are produced in mice by many chlorinated compounds, including DDT. There is also evidence to suggest that DDT metabolites DDE and TDE (DDD) produce hepatic tumours in mice and that TDE also produces lung tumours. Information on the tumorigenicity of DDT in rats (see section 7.1.9) is conflicting; some studies report tumour formation while other studies report negative data. Carcinogenicity studies in the hamster were negative (see section 7.1.9). The occurrence of tumours in some rodents only, casts doubt on the significance of the phenomenon and on extrapolation of the findings to man.

Studies on the incidence of all cancers reported in those parts of some countries with known high agricultural use of DDT in the 1950s and early 1960s have not demonstrated any trends in any type of cancer associated with the use of DDT in relation to these areas (see section 8.2.9).

The question as to whether DDT is carcinogenic in man has not been answered unequivocally. Although the cross-sectional epidemiological studies on workers exposed to DDT and the observation studies on volunteers are limited, there is not any currently available evidence to suggest that DDT is tumorigenic or carcinogenic in man (see section 8.2.9).

Recent studies on *in vitro* bacterial test systems with or without metabolic activation have not shown any evidence that either DDT or DDE is mutagenic (see section 8.2.10). The evidence for the mutagenicity of DDT in mammalian test systems is inconclusive.

9.4 Effects on Microsomal Enzymes

There is no doubt that exposure to DDT results in the induction of microsomal mixed function oxidases and causes marked morphological changes in the liver of some rodents (see section 7.1.9). In some rodents, notably the mouse, these morphological changes have been related to tumorigenicity. Microsomal enzymes are also induced by DDT in other species, but the liver does not show the same morphological changes.

The only effect for which something approaching a threshold has been demonstrated is the induction of microsomal enzymes in workers in association with an average serum value of 0.573 mg/litre for *p,p'*-DDT but not in workers with serum levels as high as 0.052 mg/litre, a value essentially identical to the highest reported for the general population. Furthermore, although some groups of workers experienced an increase in their average enzyme activity, no person exceeded the range of activity found in normal people in the general population.

DDT will not induce liver microsomal enzymes in the general population because their intake of the compound is so much less than the smallest dosage capable of producing this effect in animals or man (see sections 7.1.4.2 and 8.2.5.1).

9.5 Reproduction and Teratogenicity

Effects on reproduction in mammals have been studied in the mouse, the rat, and the dog (see section 7.1.7). In the mouse, a multigeneration study at dietary levels of DDT of 25, 100, and 250 mg/kg showed effects on fertility and reproduction only at the highest level, equivalent to 33 (mg/kg)/day. In the rat, normal reproduction was maintained at a dietary level of 200 mg/kg. In the dog, dietary intake at dosages up to 10 (mg/kg)/day did not produce any effects on reproduction other than earlier estrus in the DDT-treated females.

In man, there is no indication that DDT affects reproduction (see section 8.2.7); no impairment of fertility was observed in a study of men occupationally-exposed for more than 10 years to a measured average daily intake in the region of 18 mg/man per day (equivalent to 0.25 (mg/kg)/day).

Studies in the mouse, the rat, and the dog have not shown any evidence of teratogenicity. In the mouse, dosage at the rate of 1 mg/kg was not teratogenic; a single dosage of 25 mg/kg or repeated doses at the rate of 2.5 mg/kg were embrytoxic but not teratogenic.

9.6 Immunosuppression

DDT appears to have a depressant effect on the immune system although the evidence is by no means conclusive. Rats and rabbits receiving DDT in aqueous suspension at a concentration of 200 mg/litre showed a depression in antibody formation and decrease in at least one globulin fraction of the blood. Rats receiving a dosage of 0.25 (mg/kg)/day by gavage did not show any changes in the phagocytic activity of the white blood cells. In the

guineapig, dosages of 1–20 mg/kg did not have any effects on antitoxin production but produced a reduction in tissue histamine levels (see section 7.1.1).

9.7 Nutritional Effects and Other Factors

Animal studies indicate that nutritional status influences the toxicity of DDT (see section 7.3.3). The preferential storage of DDT in fat can mitigate the effect of acute poisoning. If rats that have stored large amounts of DDT are starved, they may suffer toxic effects due to mobilization of fat and DDT.

In man, nutritional status will have a similar effect to that found in other animals. However, the possibility that starvation in man could precipitate toxic manifestations is regarded as unlikely as the stored levels do not approach those found in laboratory animals and the lower metabolic rate of man results in slower mobilization. In fact, severe weight loss sometimes does cause some increase in storage of DDT in connexion with certain wasting diseases; however, people with full-time occupational exposure to DDT average 10 times more storage than the highest values reported in connection with disease but do not exhibit predisposition to the diseases in question (see section 6.2.1.3).

Although young animals are often more susceptible to toxic chemicals than adults, there is no evidence that DDT is more toxic to young animals of any species including man. In fact, in the rat, the young are less susceptible to a single dose than the adults (see section 7.3.2, Table 20).

9.8 Dosage-Effect Relationships

Dosage-effect relationships for DDT in man have been observed in connection with acute poisoning (see Table 23), excretion, and storage (see Fig. 4), and the induction of microsomal enzymes, which has been observed at a dosage of 0.25 (mg/kg)/day but not at lower dosages. The dosage of 0.25 (mg/kg)/day to which workers have been exposed for 25 years is of the same order of magnitude that causes an increase in tumours in male mice of a susceptible strain but not in females of any strain (see section 7.1.9). As shown in Table 24, this dosage in workers is less than the no-effect levels for rats, dogs, and monkeys and far less than the dosage at which rats, mice, and dogs successfully reproduce for generations. The equilibrium levels of DDT and its metabolites found in the blood and fat of people with full-time occupational exposure and the much lower levels found in the general population have not been associated with any adverse effects.

Table 24. Dosage-effect of DDT in animals

Single dose (mg/kg)	Route	Observation
3000	dermal	LD_{50} of powder in adult rat
2356–4000	oral	LD_{50} of oil solution in newborn rat
250–500	dermal	LD_{50} of oil solution in adult rat
250	oral	LD_{50} of oil solution in adult rat
Repeated Exposure (mg/kg)/day		
300	subcutaneous	inhibition of testicular growth in cockerels
41–80	oral	increased mortality in rats, 2-year study
41–80	oral	100% mortality in dogs in 39–49 months
41–80	oral	100% mortality in monkeys, 70 days
21–40	oral	25% mortality in dogs in 39–49 months
33.2	oral	harmful to reproduction in mice
13.3	oral	slight reduction in lactation and survival of some but not all generations of mice (6 generation test)
10	oral	no harmful effect on reproduction in dog (3 generation test)
10	oral	no harmful effect on reproduction in rat (2 generation test)
5–10	oral	no-effect level in dog, 2 generations
2.6–5	oral	no-effect level in monkey, 7.5 years
0.63–1.25	oral	no-effect level in rat, 2 year test
0.16–0.31	oral	risk of liver tumours doubled in male mice but no effect in female
0.3	oral	no-effect level for induction of microsomal enzymes in rats

9.9 Recommendations on Levels of Exposure

The data from intake, exposure, and levels in populations supports the current conditional ADI for DDT, which affords a considerable margin of safety.

If the total intake of DDT from food and other sources rises above 0.005 (mg/kg)/day (the conditional ADI) then the situation should be investigated.

The concentration of DDT in the air in industrial, agricultural, or disease control areas should not exceed 1 mg/m^3 on a time-weighted basis (40 h per week). Several countries have their own standards that range from 0.1 to 1 mg/m^3, which seem to afford an acceptable margin of safety.

There is ample reason to predict the continuing safety of workers producing and using DDT. No harmful effect has ever been reported in vector control operators who have applied DDT during the last 3 decades in public health programmes. Nevertheless, as in the case of any chemical, occupational safety and health measures should always be applied to ensure that contact with DDT by workers is kept to a minimum.

The only index of exposure of DDT or its metabolites is the analysis of

these compounds in tissues or excreta. For most purposes it is best to sample serum or plasma. In subjects with relatively constant, prolonged exposure, concentrations of DDT and its metabolites in the blood are in equilibrium with those in all other tissues, including adipose tissue. In subjects who have accidentally received a single large dose, the concentration in the brain is reflected more accurately by a serum sample than by a fat sample.

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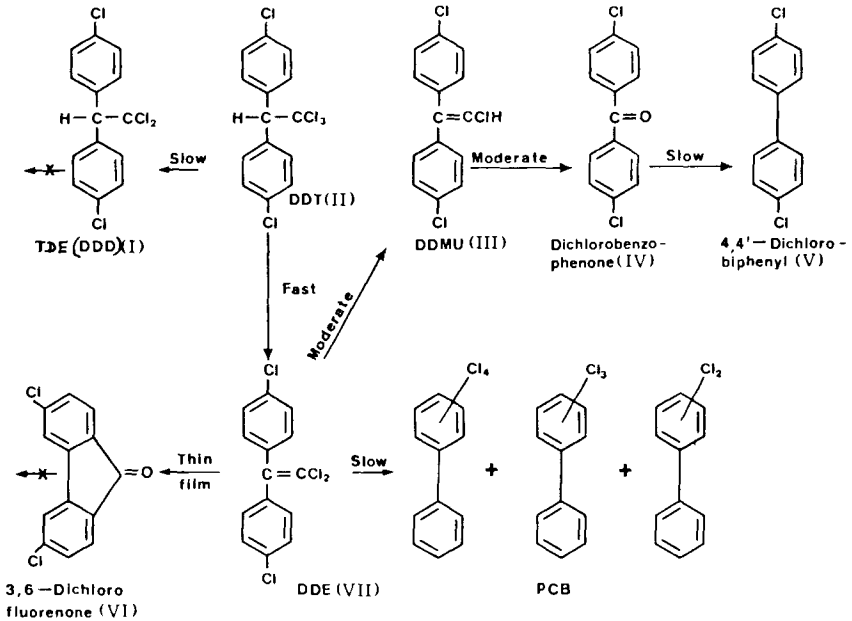
TRANSFORMATION OF *P,P'*-DDT IN THE ENVIRONMENT[a]

1 Abiotic Transformations

Since 1969, the photolysis of *p,p'*-DDT (Annex Fig. 1, formula II) and of its known primary environmental degradation product, DDE (1,1-dichloro-2,2-bis [*p*-chlorophenyl]ethylene; Annex Fig. 1, formula VII) has been



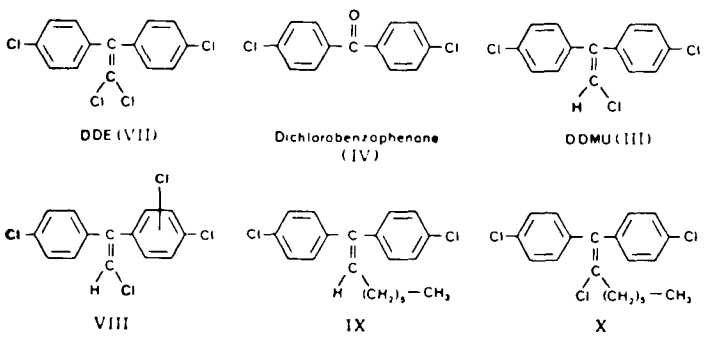
ANNEX Fig. 1. Proposed scheme for the degradation of DDT vapour in sunlight.

studied by irradiation in methanol at 260 nm (Plimmer et al., 1970). The products formed were DDMU (1-chloro-2,2-bis [*p*-chlorophenyl] ethylene; Annex Fig. 1, Formula III), dichlorobenzophenone (Annex Fig 1, formula IV) and dichlorobiphenyl (Annex Fig. 1, Formula V). The formation of the last compounds proceeded via dichlorobenzophenone as an intermediate. The detection of the chlorinated biphenyl raised the question as to whether DDT might be a source of PCBs in the environment. Upon investigation of

[a] Prepared by Dr F. Korte at the request of the Task Group.

the reaction pathways leading to these substances, it was shown that DDE is converted to 3,6-dichlorofluoroenone (Annex Fig. 1, formula VI; Plimmer & Klingebiel, 1969) which is photooxidized to 3,3'-dichlorobiphenyl-2-carboxylic acid. Subsequent decarboxylation of this acid could yield traces of 3,3'-dichlorobiphenyl; the decarbonylation of another photolysis product of DDT, trichlorobenzophenone, could yield traces of trichlorobiphenyl, demonstrating that the formation of PCBs with more than 2 chlorine atoms was also possible (Plimmer & Klingebiel, 1973).

Irradiation studies with substances in organic solvents are not necessarily predictive for the environment. However, studies with DDT vapour in sunlight confirmed the results obtained with dissolved substances. The proposed pathways of DDT photolysis under environmental conditions is shown in Annex Fig. 1 (Moilanen & Crosby, 1973).



ANNEX Fig. 2. Reaction products of DDE upon irradiation with ultraviolet light under various conditions.

In 1972, DDE was irradiated in solvents, in the solid state, and in the gaseous phase, with UV-light of various wavelengths. The results are shown in Annex Fig. 2. Besides the known photoproducts IV and III, a "trichlorinated DDMU" (Annex Fig. 2, formula VIII) and 2 compounds with longer side chains (Annex Fig 2, formula IX and X) were identified; these 2 substances, however, were formed only upon irradiation in a solvent and originated from the reaction with the solvent (Kerner et al., 1972).

In a recent study on the photoisomerization and photodegradation of DDE under simulated natural conditions (inert solvents, a good hydrogen donor solvent, UV-light $\simeq$ 300 nm), the compound VIII (Annex Fig. 2) with the 3 phenyl-bound chlorine atoms was detected and characterized as a mixture of the E- and Z-isomers which were separated and isolated. Both isomers were also found in natural samples like tobacco and pine needles. DDMU was also detected in these studies and 2 substances so far unknown, a tetrachlorinated phenanthrene and a tetra-ring-chlorinated diphenyl-

ethylene; the formation of tri- and tetrachlorobiphenyls was confirmed (Göthe et al., 1976).

The behaviour of compounds in their adsorbed form is equally as significant environmentally as their photochemical behaviour in the gaseous and solid states.

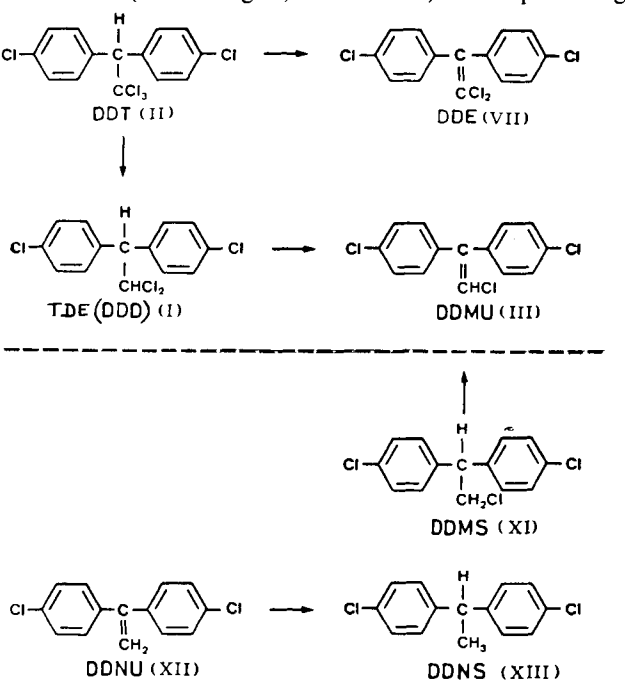
Irradiation of DDE, adsorbed on silicagel, with wavelengths > 230 nm, resulted in the formation of dichlorobenzophenone and its trichlorinated analogue. Irradiation of DDT and DDE in the solid form in an oxygen stream with wavelengths > 230 nm, resulted in partial mineralization to give carbon dioxide and hydrochloric acid (Gab et al., 1975).

The results presented here show that a large number of DDT-derived chlorinated compounds must be included, when considering the possible effects of DDT residues in the ecosphere.

2 Biotransformations Other Than Mammalian Metabolism

2.1 Birds

Two main pathways of DDT metabolism exist in mammals i.e., dehydrochlorination to DDE (Annex Fig. 3, formula VII) and stepwise degradation



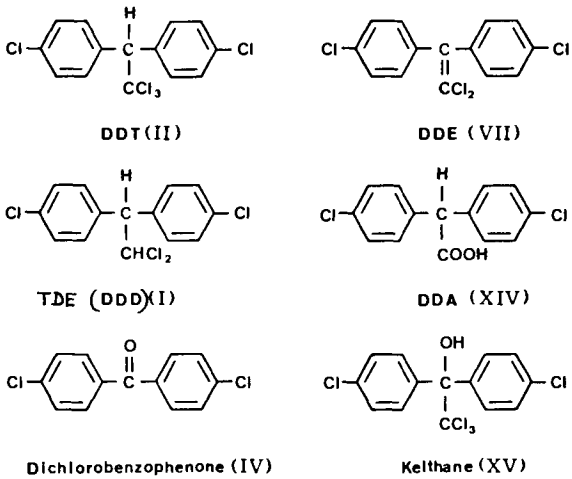
ANNEX Fig. 3. Biotransformation of DDT in the pigeon.

to DDA (bis-[*p*-chlorophenyl] acetic acid) via TDE (DDD) (1,1-dichloro-2,2-bis [*p*-chlorophenyl] ethane; Annex Fig. 3, formula I). However, in birds, the pathway varies with species, and data from studies on the administration of chronic and acute dosages of DDT to pigeons, quail, and blackbirds show that DDE is the primary metabolic product in the first 2 species, and TDE (DDD) in the third (Bailey et al., 1972). The TDE-pathway does exist in the pigeon as a minor pathway but, in contrast to mammals,only as far as DDMU (Annex Fig. 3, formula III).

Thus, DDA, the degradation product of DDMU excreted by mammals, is not formed in the pigeon (Bunyan et al., 1966; Bailey et al., 1969). When its precursors in mammals, DDMS (1-chloro-2,2-bis[*p*-chlorophenyl] ethane; Annex Fig. 3, formula XI) and DDN U (1,1-bis[*p*-chlorophenyl] ethylene; Annex Fig. 3, formula XII) are administered to the pigeon, they are rapidly converted: DDMS is converted to DDMU, and DDNU is metabolized quickly and excreted as DDNS (1,1-bis [*p*-chlorophenyl] ethane; Annex Fig. 3, formula XIII), a metabolite that was not found in mammals (Bailey et al., 1972). The metabolic pathways for DDT in the pigeon are shown in Annex Fig. 3.

2.2 Insects

Investigations on the detoxication mechanism of DDT in insects are interesting as regards the problem of resistance. In general, the phenomenon of insect resistance is related to detoxication of the insecticide by metabolization to nontoxic compounds. The metabolic pathways of DDT in insects are many and depend on species and even on strains.

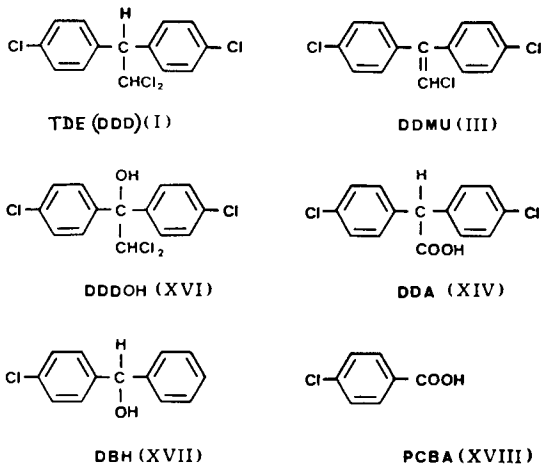


ANNEX Fig. 4. Conversion products of DDT in insects.

Annex Fig. 4 shows only the major metabolic products.

The first conversion product identified in resistant houseflies was DDE. This conversion is catalyzed by the enzyme DDT-dehydrochlorinase (EC 4.5.1.1) which had already been isolated in the pure form in the 1950s. Further insect metabolites of DDT are DDD (isolated for example from *Stomoxys calcitrans*), DDA (isolated from example from *Quiscalus quiscula*, *Heliothis virescens* and *Coleomegilla maculata*) and dichlorobenzophenone (from *Leucophae*). The detoxiation of DDT in *Triatoma infestans*, *Drosophila melanogaster*, *Culex tarsalis*, and other species is performed by hydroxylation and results in kelthane (Annex Fig. 4, formula XV), a substance which is a commercial acaricide. A great number of unidentified and water-soluble conversion products of DDT was observed in many species as reviewed by Klein & Korte (1970).

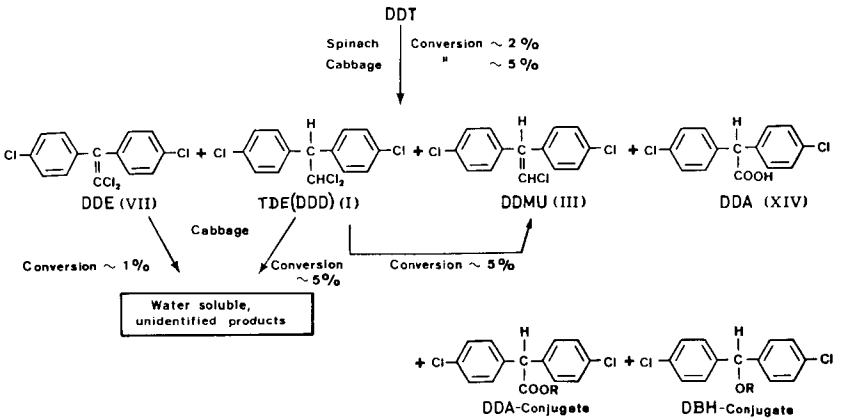
Although TDE (DDD) has not been found as a DDT-metabolite in *Culex tarsalis*, it has been concluded from differences between susceptible and resistant strains that it is an intermediate in the further degradation of DDT. After application of TDE ^{14}C to this insect, DDMU and DDDOH (1,1-bis[*p*-chloropheyl]-2,2-dichloroethanol; Annex Fig. 5, formula XVI) were found as major metabolites; furthermore, 3 polar compounds were chromatographically identical with DDA, DBH (dichlorobenzhydrole; Annex Fig. 5, formula XVIII) and PCBA (*p*-chlorobenzoic acid; Annex Fig. 5, formula XVIII), which were also observed after application of DDMU-^{14}C (Plapp et al., 1965). The occurrence of PCBA indicates the complete breakdown of one of the 2 rings of DDT and thus the possibility of a complete biological degradation of the whole molecule.



ANNEX Fig. 5. Degradation products of TDE (DDE) in the insect *Culex tarsalis*.

2.3 Higher plants

Although the transformation of DDT in higher plants is rather limited (2% in spinach within 18 days, 5% in cabbage within 14 weeks), it must not be neglected since a considerable part of the DDT used on a worldwide basis is applied, intentionally or unintentionally, to plants. The conversion products that have been identified (Annex Fig. 6) are DDE, TDE (DDD), DDMU, DDA, conjugates of DDA, and a conjugate of DBH (Zimmer & Klein, 1972), which means that the metabolites in plants are not chemically different from those in other organisms.



ANNEX Fig. 6. Conversion of *p,p'*-^{14}C in spinach and cabbage.

In a study of the accumulation and distribution of *p,p'*-DDT in an apple orchard, DDT residues in or on the roots and leaves of the herbage and the roots, bark, leaves, and fruit of the trees were recorded for an orchard sprayed annually (Stringer et al., 1975). During 13 years, there were increasing amounts of DDE, TDE, and DDMU in relation to DDT, in the bark of apple trees indicating some breakdown on the bark (<10%). DDE and TDE were also observed after application of *p,p'*-DDT to cotton (Nash et al., 1977). These 2 substances seem to be common conversion products of DDT in plants. The *o,p'*-DDT observed in the last 2 experiments seems to be an impurity of the DDT rather than a metabolite.

2.4 Microorganisms and soil

The most common metabolic reaction of DDT in microorganisms is reductive dechlorination resulting in the formation of TDE. This reaction has been demonstrated in *Escherichia coli* (in rat intestine), *Aerobacter aerogenes*, *Proteus vulgaris*, and in yeasts. In contrast to metabolism in

higher animals, dechlorination by microorganisms is anaerobic and is catalysed by reduced cytochrome oxidase (EC 1.9.3.1). Fe (II)-cytochrome oxidase isolated from *Aerobacter* converts DDT to TDE *in vitro* (Klein & Korte, 1970). The conversion of DDT to TDE (DDD) in bodies of water (Miskus et al., 1965) and in other reducing environments characteristic of dead and decaying matter (Zoro et al., 1974) is mediated by reduced iron porphyrins and is not an essential part of cell metabolism. These findings have considerable environmental significance since most living material contains iron porphyrins bound with protein in complex molecules. The porphyrins are released after decay of the organic substances, and may then be regarded as widespread environmental agents that convert, on a larger scale, the persistent DDT to the less persistent TDE. TDE is susceptible to further abiotic or biotic degradation.

However, the formation of DDE and DDA from DDT by microorganisms is also possible. For instance, both DDE and TDE were isolated from *Serratia marcescens* and *Alkaligenes faecalis* (Stenersen, 1965) and DDA was isolated from microbial cultures obtained from agricultural soil (Patil et al., 1970).

In a model experiment with anaerobic activated sludge and *p,p'*-DDT-^{14}C, TDE, *p,p'*-dichlorobenzophenone, DDMU and a so far unknown metabolite, DDCN (bis[*p*-chlorophenyl] acetonitrile), were detected as conversion products. The last of these substances, a minor conversion product, was also found in the sediment layer of the Lake Mälaren in Sweden (0.2 mg/kg dry weight). DDCN is formed via TDE or DDE, but directly from DDT (Jensen et al., 1972).

The question of "bound residues" in soil, which is now under discussion for a number of "non-persistent" pesticides, especially those that are anilin-derived, seems also to be relevant for "persistent" substances such as DDT, although the percentage of bound residues is less than for less persistent pesticides. The formation of 25% of bound DDT-residues within 28 days (Lichtenstein et al., 1977) justifies a reassessment of the persistence of DDT in soil. Further information should be obtained concerning the nature and the potential biological activity of the compounds that are bound.

3 Conclusion

A multitude of conversion products are formed from DDT under environmental conditions. Nearly 20 of these (including mammalian metabolites) have been identified so far, but the chemical structure of a number of other compounds is still unknown. Very little is known of the toxicological properties of these conversion products with the exception of major

products such as DDE and TDE. This should be remembered when the unwanted effects of DDT in the environment are evaluated. However, there is even less information concerning the fate in the environment of many other pesticides including those that are used as DDT-substitutes.

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