LONG-TERM PROGRAMME IN ENVIRONMENTAL POLLUTION CONTROL IN EUROPE

THE HAZARDS TO HEALTH AND ECOLOGICAL EFFECTS OF PERSISTENT SUBSTANCES IN THE ENVIRONMENT - POLYCHLORINATED BIPHENYLS

Report on a Working Group

Brussels
3-7 December 1973

REGIONAL OFFICE FOR EUROPE
World Health Organization
COPENHAGEN

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Report on a Working Group convened by the Regional Office for Europe of the World Health Organization

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Note

This report has been prepared by the Regional Office for Europe of the World Health Organization for distribution to the governments of Member States in the Region and to all who participated in the Working Group on The Hazards to Health and Ecological Effects of Persistent Substances in the Environment - Polychlorinated Biphenyls, Brussels. A limited number of copies are available for persons officially or professionally concerned with this field of study from the WHO Regional Office for Europe, Copenhagen.

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This report is also available in French and Russian.

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FOREWORD AND SUMMARY

The Regional Office for Europe of the World Health Organization, in collaboration with the Government of Belgium, and under the project "Hazards to Health and Ecological Effects of Persistent Substances in the Environment", which forms part of the long-term programme in environmental pollution control, convened a Working Group in Brussels from 3 to 7 December 1973 to discuss PCBs (polychlorinated biphenyls) in the environment

Professor S Halter welcomed the participants on behalf of the Government of Belgium, Dr V Krichagin addressed the meeting on behalf of Dr Leo A. Kaprio, Regional Director, World Health Organization Professor A. Lafontaine was elected as Chairman, Professor F. Kaloyanova as Vice-Chairman and Dr A Jernelöv as Rapporteur.

The scope and purpose of the Working Group was to make a critical review of available information on long-term health hazards and ecological effects of PCBs in the environment and to discuss analytical problems in connexion with the detection and quantitation of PCBs in environmental and biological samples.

Furthermore, the Working Group was to consider the need for continuous attention to be given to PCBs in view of the steps already agreed on to reduce their use and, finally, it was to discuss possible problems in connexion with substances such as PCTs (polychlorinated tars) that are used or are being considered for use as replacements for PCBs.

Before the meeting, working papers dealing with the human health and ecological aspects of contamination by PCBs has been prepared and distributed to the participants for review and comment

Conclusions

New analytical techniques, especially those involving capillary columns, seem to offer possibilities for separation, identification and quantitation of individual chlorobiphenyls, and these may greatly increase the possibilities of understanding the medical and ecological effects of PCBs.

The interpretation of results of toxicity studies is complicated by the possible contamination of mixtures of PCBs with toxic impurities. Estimation of an Acceptable Daily Intake (ADI) for use is not possible at this time, since the composition of the mixtures of PCBs present in food differs from that of the commercial mixtures used in animal experiments.

The information at present available on levels in different parts of the biosphere is not sufficient or reliable enough to support any firm mass-balance or transport-rate calculations or assessment of large-scale ecological impact, or to provide a good basis for future trend analysis. Much more data are undoubtedly needed.

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Recommendations

Bearing in mind the most disturbing gaps in existing knowledge on this subject, the Working Group adopted a series of specific research recommendations, together with a number of a more general character

After examination of the data now available concerning health and environmental effects of PCBs, their high persistence and their present widespread distribution in the biosphere, the Working Group concluded and strongly recommended that, firstly, the use of PCBs should be limited to the few critical applications for which no substitutes are now available and in which no substitutes are now available and in which highly efficient containment and recovery are possible; secondly, that safe substitutes for these and other uses be developed, since the substitution of another toxic and persistent agent for an existing one can result only in further harm Accordingly, adequate pre-testing is imperative.

The Working Group agreed that, as a first step towards achieving this purpose, uses of PCBs that lead to environmental contamination should be avoided, along the lines of the decisions and recommendations of the OECD[1] These should be extended to all countries, and further steps should be taken.

Member States should ensure that, in their respective territories, PCBs will not be used for industrial or commercial purposes, except in the following categories of use:

- dielectric fluids for transformers or large power factor correction capacitors;
- heat transfer fluids (other than in installations for the processing of foods, drugs, animal feeds and veterinary products);
- hydraulic fluids in mining equipment;
- small capacitors;

and, as far as the above categories are concerned, PCBs may be used only in those applications in which the requirements for non-inflammability out-weight the need for environmental protection and regarding which Member States are satisfied that sufficient controls are exercised in order to minimize risk to the environment.

[1] Adopted by the Council of the Organisation for Economic Co-operation and Development (OECD) at its 315th meeting, 13 February 1973

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Substitutes should be sought and carefully studied before use, in order to detect possible toxicological effects on human health and the environment. Substitutes already in use should be examined for their adequacy and safety and should be withdrawn if necessary. The concept of pretesting should be applied to all new chemicals developed for widespread use.

Analytical techniques should be developed to ensure the detectability, at levels causing non-admissible harmful affects, of any technical chemical that can accumulate in biological media

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1. INTRODUCTION

Polychlorinated biphenyls (PCBs) were introduced into commerce in 1929. Although there were sporadic reports during the 1930s and 1940s in the industrial medical literature of toxic effects of PCBs and of mixtures containing PCBs, they were not generally regarded either as hazardous chemicals or as potential environmental contaminants. Although it now appears that they were commonly present in specimens analysed for chlorinated hydrocarbon pesticides in the 1950s and 1960s, they were ignored or dismissed as "unknown interfering compounds" until they were identified by Jensen and Widmark in 1966. However, they attracted little general concern until a series of incidents drew attention to their wide distribution and potential hazard. The most serious such incident was the large-scale contamination of cooking oil in Japan in 1968; this affected more than 1000 persons, many of whom suffered severe and persistent skin lesions. The same accident led to contamination of chicken feed, causing large-scale mortality of chickens; a similar case in which chickens were affected occurred in the United States of America in 1971. A report of large-scale mortality of fish-eating birds in the United Kingdom in 1969 was accompanied and followed by the discovery of toxicologically significant levels of PCBs in fish used for human consumption in many different countries. These incidents have led to a rapid increase in the number of studies on the distribution and effects of PCBs.

Gas-liquid chromatography, often in conjunction with mass spectrometry, nuclear magnetic resonance, and infra-red spectrometry, has been used to determine the composition of the major commercial products. The probable structures of the principal isomers in the commercial mixtures (as many as 78 for Aroclor 1260) have been determined. There remains a need, however, for standardization in the labelling of peaks in chromatograms on environmental samples in order to permit the identification of the components with known isomers in the commercial products. The early studies of the properties of the PCBs were focused on the determination of the electrical, thermal, and chemical properties associated with their industrial application. Little is known, however, of the partitioning of PCBs between aqueous solutions and lipids, particulates and sediment. Information on the fractionation of isomers during transfer between phases is fragmentary.

Production statistics and quantitative patterns of use for the United States of America released recently by Monsanto show a peaking of sales in 1970 at 33×10^6 kg. The major uses, in order of importance, prior to 1970 were for capacitors, plasticizer applications, including use in carbonless duplicating paper, transformer fluids, hydraulic fluids and lubricants, and heat-transfer fluids. Sales, subsequently restricted by Monsanto mainly to electrical applications, declined to 15×10^6 kg in 1971 and were projected at 13×10^6 kg for 1972. Total sales in the United States of America since the introduction of PCBs are estimated to be 5×10^5 tons.

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PCBs are also manufactured in Czechoslovakia, France, the Federal Republic of Germany, Italy, Japan, Spain, the USSR, and the United Kingdom. Data on production and use for these countries are not available, but it is expected that the use and loss patterns will have been broadly similar in all industrialized countries. As in the case of most industrial chemicals, loss figures for PCBs are practically non-existent. Rough estimates of losses to the environment, based on the nature of the application and the physical properties of PCBs, suggest that approximately 20% of the cumulative production is still in service in transformers, heat exchangers, and capacitors, 5% has been discharged into the atmosphere by vaporization of plasticizers and in burning dumps, 10-15% has been discharged into fresh or coastal waters by disposal of hydraulic fluid and lubricant plus small amounts of heat-transfer and transformer oils, and 55% has been disposed of in dumps and incinerators. Of the latter, it is estimated that 10-20% was destroyed by burning.

The transport of the PCBs through the atmosphere is expected to be similar to that of DDT and its metabolites. PCBs, discharged mainly in urban and industrial areas, are expected to be carried by air-borne particulates. Most of the PCBs are deposited near their source, but some are transported on fine particulates to remote locations. The deposited PCBs are vaporized and redeposited, eventually reaching the oceans. By analogy with DDT, it is estimated that roughly one-quarter of the PCBs released into the atmosphere have been deposited in the oceans.

The PCBs released into surface water are expected to be highly adsorbed by the bottom sediment, and to be transported downstream at a low rate, mainly by water-borne particulates. The major routes of PCBs into estuaries and coastal waters are expected to be via sewage, the dredging of inland rivers and harbours, and ocean dumping of industrial chemicals. The total cumulative input into the oceans around North America by both localized discharge and aerial fall-out is estimated to be of the order of 1.5×10^4 tons.

From the differences between the ratios of isomers in the environmental samples and the commercial PCBs, it is tentatively concluded that the tetrachlorobiphenyls and the low-chlorine forms are decomposed, probably by microbial action in sediments, and that certain higher isomers are photochemically degraded during transport.

As yet, little is known of the distribution of PCBs in terrestrial environments. No studies have been reported of the uptake of PCBs from the soil by soil fauna or by plants, from plants by herbivores, or from herbivores by carnivores. There are a few reports of direct measurements of levels of PCBs in air or on air-borne particulates.

A number of studies have been made of the uptake of PCBs by aquatic animals, both from contaminated sediments and from solution or suspension in water. Both invertebrates and fish accumulate PCBs up to levels 10^3-10^5 times as great as those in the ambient water.

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There is further concentration within vertebrate food chains, so that levels in top predators (birds, sharks, seals, etc.) may be 10^7-10^8 times higher than those in the ambient water. The distribution of residues of PCBs in birds and fish of both fresh- and salt-water environments indicates high-level contamination in areas near industrial centres, in addition to widespread low-level contamination. Fish populations with mean levels of 1 mg/kg of PCBs have been reported in various European waters, off the north-east coast of North America, and in bays and estuaries of the Gulf of Mexico, California, and Japan. These levels may be of toxicological significance to predators of these populations. Much higher levels (10-800 mg/kg) have been reported in fish from highly contaminated inland waters (the Great Lakes and certain industrialized rivers in North America, Lake Biwa in Japan). Levels of PCBs appear to be 5-10 times higher in animal populations from the North Atlantic Ocean than in those from the North Pacific. Levels are reported to be remarkably high in plankton from the North Atlantic, perhaps indicating contamination of lipid-rich surface slicks. Few data are available from the southern hemisphere.

Most exposure of the general human population to PCBs appears to be by ingestion of fish in the diet. Accordingly, an average intake of the order of 5-20 µg/day is likely for the average adult person in the United States of America, but this will probably vary greatly according to location and dietary preference. Sporadic instances of contamination of other foods (e.g., milk and poultry) may possibly have led to significantly higher intakes for short periods. Breast-fed infants ingest significantly higher doses per unit body weight than adults. Mean levels of PCBs recorded in human fat are of the order of 1 mg/kg in the United States of America, but of the order of 6 mg/kg in the Federal Republic of Germany.

Toxicological studies of PCBs are complication by the fact that they consist of mixtures of many isomers with potentially different toxicities. Studies have been further complicated by the recent discovery that two batches of commercial PCBs (manufactuted in France and the Federal Republic of Germany) contained highly toxic contaminants, almost certainly chlorinated dibenzofurans (CDBFs). CDBFs have not been identified in environmental samples, but may be toxicologically significant at levels too low (sub-nanogram) for detection by existing techniques. There are indications that different batches of PCBs vary in toxicity to chickens, and it is conceivable that CDBFs may be produced from PCBs in the environment. This problem should be given high priority in future studies.

Work on uptake, retention, and excretion of PCBs is still incomplete. Very little work on metabolism has been done, and no metabolites have so far been identified precisely, although ring-hydroxylated derivatives were reported in one study. PCBs are accumulated slowly by vertebrates; in one study on rats, equilibrium levels had not been reached after 240 days of continuous exposure. Loss of PCBs from the body after cessation of exposure may be more rapid. There is circumstantial evidence that lower PCBs (tetrachloro and lower isomers) are metabolized fairly rapidly in the environment. Higher PCBs are much more refractory.

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but some pentachloro and hexachloro isomers appear to be metabolized by birds and mammals

PCBs freshly manufactured in the United States of America have comparatively low acute toxicity to the birds and mammals that have been tested In long-term tests of low levels of exposure, a number of different effects have been observed. Among the most consistent are enlargement of the liver and induction of hepatic microsomal enzymes. A number of different effects on reproduction have been described, including reduced mating indices in rats, embryotoxicity in rabbits and birds, and oestrogenic or anti-androgenic effects on rats and birds. Some of these effects increase with increasing chlorine content of the mixture, while others decrease Only preliminary experiments have been conducted with single isomers. It seems likely that some of the observed effects, such as enzyme induction oestrogenic effects and perhaps porphyria, should be attributed to PCBs or their metabolites, others, however, such as chloracne, hydropericardium, and perhaps some of the embryotoxic effects, should probably be attributed to CDBFs. One experiment indicated immunosuppressive effects. As yet, there is no evidence of teratogenic effects in mammals, testing for carcinogenicity and mutagenicity is incomplete, and early experiments have given conflicting results.

PCBs are moderately toxic to fish, but are toxic to some aquatic invertebrates at levels as low as 0.001 mg/kg in ambient water. They inhibit growth and photosynthesis of phytoplankton at levels of 0.01-0.1 mg/kg. They are comparatively non-toxic to terrestrial insects, but act synergestically to increase the toxicity to insects of a number of pesticides.

In the "Yusho" incident, in which mass human poisoning occurred in Japan, PCBs appear to have caused severe symptoms at levels of the order of 200 µg/kg day, lower than the lowest levels at which adverse effects have been reported in experimental mammals. However, the symptoms, including chloracne and eye discharge, suggest that CDBFs may have been a major causative agent.

Levels of PCBs in some of the more highly contaminated ecosystems exceed those required to cause adverse effects on reproduction and survival of representative animal species in the laboratory. Adverse effects on wild populations of crustaceans, fish and birds appear likely, but are difficult to demonstrate in the presence of other pollutants

2 CHEMICAL COMPOSITION AND PROPERTIES

2.1 Chemical and physical properties of components

The commercial mixtures of PCBs (table 1) are colourless, viscous liquids with a solubility in water ranging from 50 to 200 µg/litre, and

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decreasing with increasing chlorine content. The molecular weights of compounds in the mixtures range from 154 to 460 Consequently, the vapour pressures (volatilities) differ by four orders of magnitude and the solubility in water varies by a factor of eight. PCBs are soluble in fats and fat solvents They are very heat stable and are not destroyed below 1300°C in a normal furnace. PCBs do not conduct electricity and impart many desirable properties to many synthetic polymers. They are not decomposed by strong acids or alkalies. These remarkable properties are responsible for their multitude of industrial uses and for their stability in the environment.

Table 1

TRADE NAMES AND CHLORINE CONTENT OF SOME POLYCHLOROBIPHENYL MIXTURES

Trade name	Country of manufacture	Chlorine content (%)
Aroclor 1242	USA	42
Aroclor 1254	USA	54
Aroclor 1260	USA	60
Clophen A60	Fed. Rep. of Germany	60
Kanechlor 400	Japan	48
Phenoclor DP6	France	60

2.2 Purity of products

Dr S. Jensen[1] summarized briefly the production chemistry of PCBs and the complexity of the spectrum of mixtures of compounds produced Table 2 gives the structural formulae of some of the compounds together with that of a polychlorodibenzofuran, a by-product of the manufacturing process often present in the mixtures used and suspected of being responsible for some of the extreme toxicities observed (Vos, 1972).

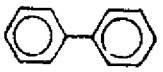
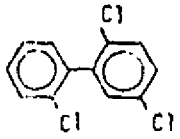
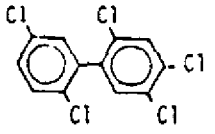
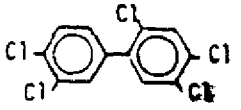
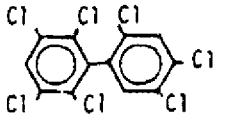
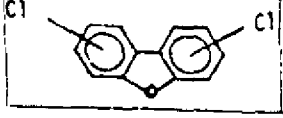
As is well known, PCBs consist of a large number of chlorinated biphenyls. These are manufactured by passing chlorine through melted biphenyl. By stopping the process after the uptake of definite amounts of chlorine, PCBs with 20, 30, 40, 50 and 60% chlorine are manufactured. The 50% fraction consists mainly of pentachlorobiphenyl, but also contains tetra-, hexa- and heptachlorobiphenyls

[1] Working paper presented to the Working Group

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Table 2

STRUCTURAL FORMULAE AND CHLORINE CONTENT OF BIPHENYL, SOME POLYCHLOROBIPHENYLS AND IMPURITIES PRESENT IN COMMERCIAL MIXTURES OF PCBs

Structure	Name	Chlorine content (%)
	Biphenyl	0
	2, 5, 2'-Trichlorobiphenyl	41
	2, 4, 5, 2', 5'-Pentachloro-biphenyl	54
	2, 4, 5, 3', 4'-Pentachloro-biphenyl	54
	2, 4, 5, 2', 3', 5', 6'-Heptachlorobiphenyl	62
	a Polychlorodibenzofuran	-

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The total number of chlorobiphenyls theoretically possible is 210, but if the chlorine is distributed between the two phenyl rings as evenly as possible, the number decreases to 104. Not all these chlorobiphenyls exist, however, in the mixtures of PCBs. The first chlorine atom will practically always enter the biphenyl in the 2- or 4- position, so that either of these will always be substituted. The second chlorine atom will usually enter the ortho- or para-position to the first. Sissons and Welti (1971) identified at least 80 different chlorbiphenyls when using a support coated open tubular (SCOT) column coated with Apiezon L in a combined mass spectrometer-gas chromatograph. Schulte and Acker (1974) described the use of capillary columns coated with SE30 for the separation of chlorobiphenyls in Clophen A60 and biological materials. Up to 75 peaks were obtained from Clophen A60. As most environmental samples show patterns of PCBs similar to those of the 50-60% chlorinated preparation, the maximum number of chlorobiphenyls we have to deal with is in the neighbourhood of 40 individual compounds, but may often be lower.

In the animal experiments described, mixtures of PCBs were used that differed in average percentages of chlorine. In addition, the isomeric composition of mixtures with the same percentage chlorine (but from different companies) are not necessarily the same. Comparison of experimental findings is therefore difficult. Apart from these differences in the composition of the mixtures, probably a more serious problem is that of the presence of toxic impurities in PCBs, a situation analogous to the presence of 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin as a toxic impurity in commercial chlorophenols and their derivatives. Vos and Koeman (1970) and Vos and Beems (1971) found marked differences in toxicity between three commercial preparations of PCBs with approximately the same degree of chlorination A Phenoclor DP6 and Clophen A60 sample was much more toxic to chicks and rabbits than an Aroclor 1260 sample. These three mixtures were fractionated by column chromatography, and most of the toxicity in a chick-embryo assay was traced to the more polar third fractions of Clophen A60 and Phenoclor DP6 (Vos et al., 1970). Mass-spectrometric analysis of these third fractions revealed identical chlorinated compounds in Clophen A60 and Phenclor DP6, but not in Aroclor 1260. From the fragmentation patterns, some of these compounds were identified as tetrachlorodibenzofuran and pentachlorodibenzofuran, estimated upper limits for the concentration of the pentachlorodibenzofuran were 5 mg/kg in Clophen A60 and 20 mg/kg in Phenoclor DP6 (the lower limit of detection was 1 mg/kg). Recently, Bowes et al. (1973) confirmed and extended these findings using improved analytical procedures; they demonstrated the presence of 2-tetra, penta, and hexachlorodibenzofuran isomers in the Clophen A60 and Phenoclor DP6 samples.

Tri- and tetrachlorodibenzofuran was found to be extremely toxic (Bauer et al., 1961); mortality in rabbits and acnegic lesions on the rabbit ear could be produced with doses that were approximately 10 times higher than for the extremely toxic 2, 3, 7, 8-tetrachlorobenzo-p-dioxin. For this reason, proper evaluation of toxicity and residue data for PCBs is complicated by the possible presence of toxic impurities in commercial preparations

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2 3 Analytical problems

A technical product such as Clophen A50 or Aroclor 1254 contains, as already mentioned, approximately 50 % of chlorine and the number of chlorine atoms per molecule usually varies over the range 4-8. The corresponding conventional gas chromatogram obtained with a column material such as a mixture of methyl and fluorinated methyl silicones shows 14 peaks (see fig. 1(a)). In contrast, capillary gas chromatography may yield up to 75 peaks (Schulte and Acker, 1974). In the conventional system (fig. 1(a)), peak number 3 consists of three isomeric penta-compounds (Jensen and Sundström, 1974). Many of the other peaks are produced by mixtures of several chlorobiphenyls. Some minor components in commercial mixtures may be particularly stable and could therefore become of greater importance in the environment as a result of progressive accumulation, such components must therefore be taken into account

The conventional gas chromatographic column cannot separate all the chlorobiphenyls from one another nor can it separate PCBs from DDT and its metabolites DDD and DDE, usually present in biological samples If it is remembered that the o,p-isomers may also be present, there are, in addition to the PCBs, another 6 substances whose peaks appear within the same range of retention times as the 10 main components of PCBs. Fig. 1 shows chromatograms of: (a) a commercial mixture of PCBs (Clophen A50), (b) DDT and its metabolites; and (c) a mixture of PCBs and DDT and its metabolites, the last being a most complicated chromatogram. Fig. 2 shows two chromatograms of biological samples. The PCBs in the herring sample (a) are difficult to quantify due to the high content of DDT and its metabolites, while in the plankton sample (b), DDT and its metabolites are difficult to estimate because of the high content of PCBs. The difficulties encountered with such complex mixtures bring out the need for preliminary treatment of the samples before gas chromatographic analysis to achieve a partial separation of the residues of PCBs from other contaminants. It may also be possible to solve this problem by means of capillary columns (Schulte and Acker, 1974) (fig. 3).

To improve the results obtained by conventional analysis, a description is given below of the determination of PCBs step by step; the different alternatives are pointed out. As DDT and its major metabolites DDD and DDE are the pollutants that most often interfere with PCBs, the discussion is mainly concerned with the problems arising from such interference.

2 4 Analysis

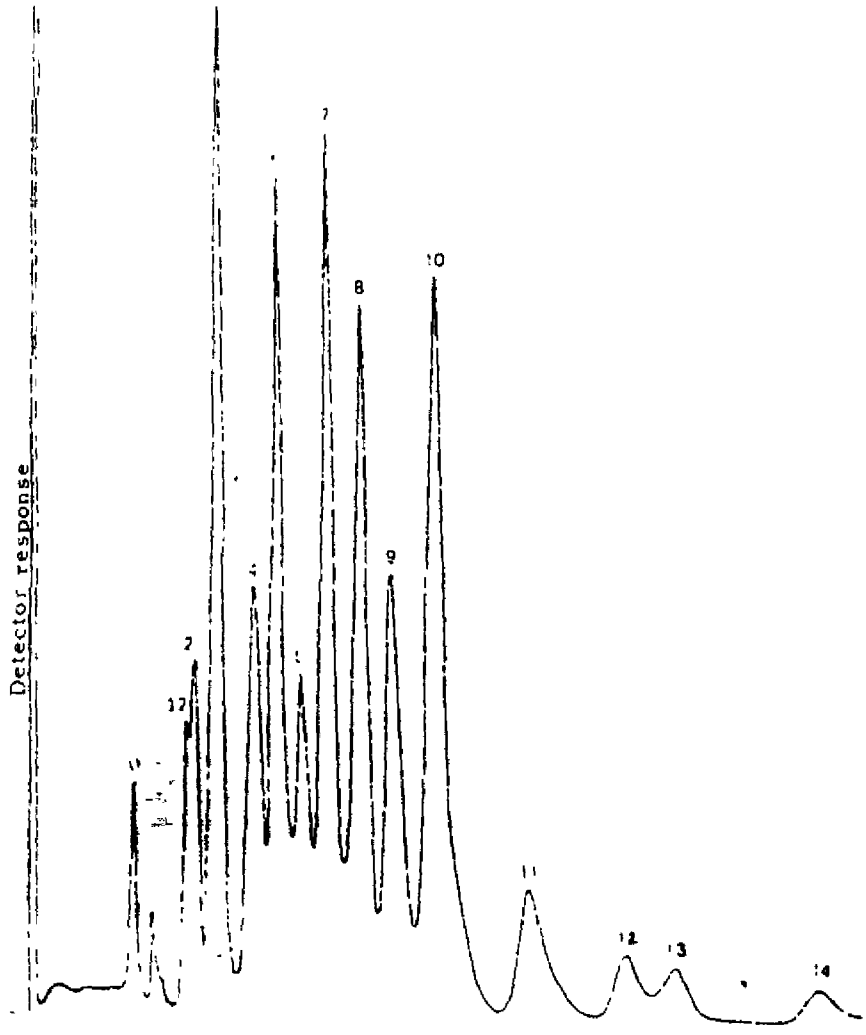
The conventional analysis consists of five main steps (1) extraction, (2) clean-up; (3) fractionation or derivatization, (4) gas chromatography, and (5) quantitation.

The first two stages will depend to some extent on the nature of the sample being analysed.

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Fig 1(a)

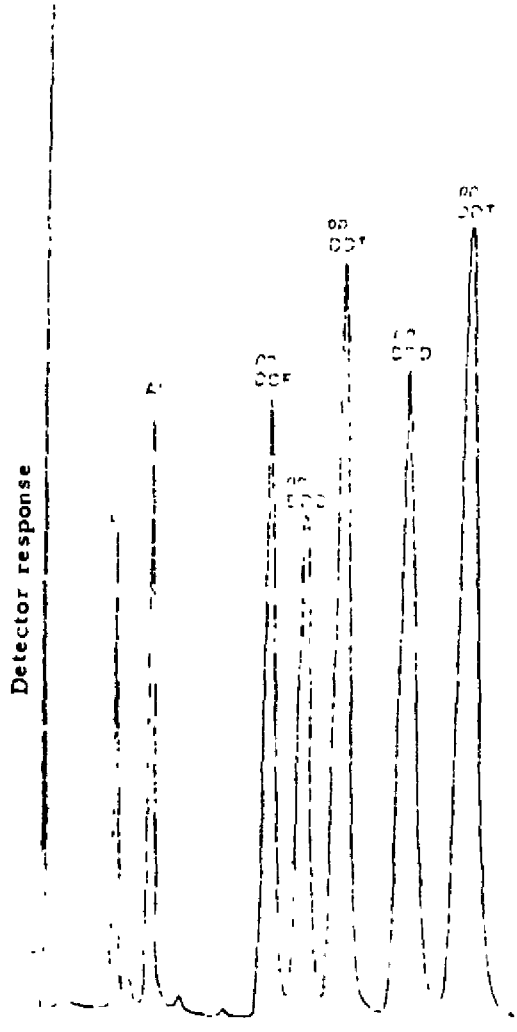
CHROMATOGRAM OF PCBs (CLOPHEN A50)



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Fig. 1(b)

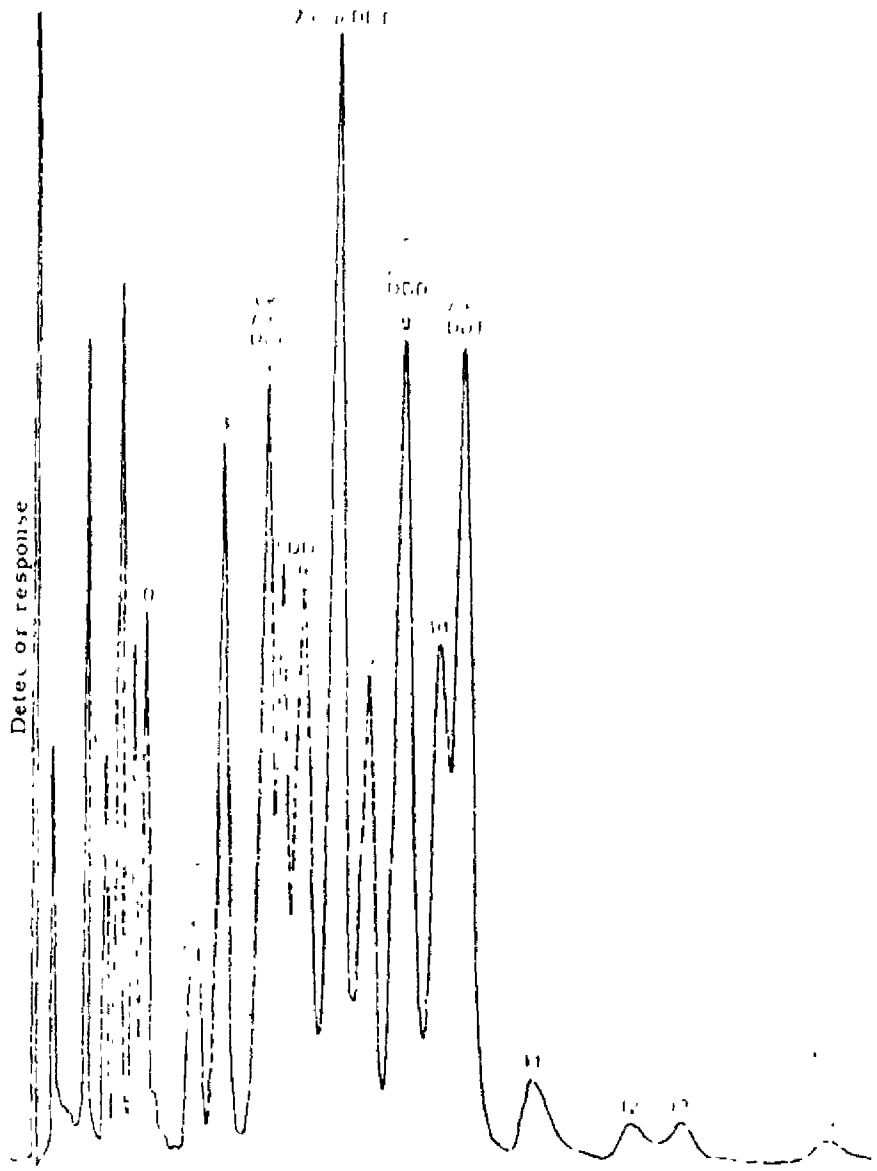
CHROMATOGRAM OF DDT AND ITS METABOLITES



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Fig 1(c)

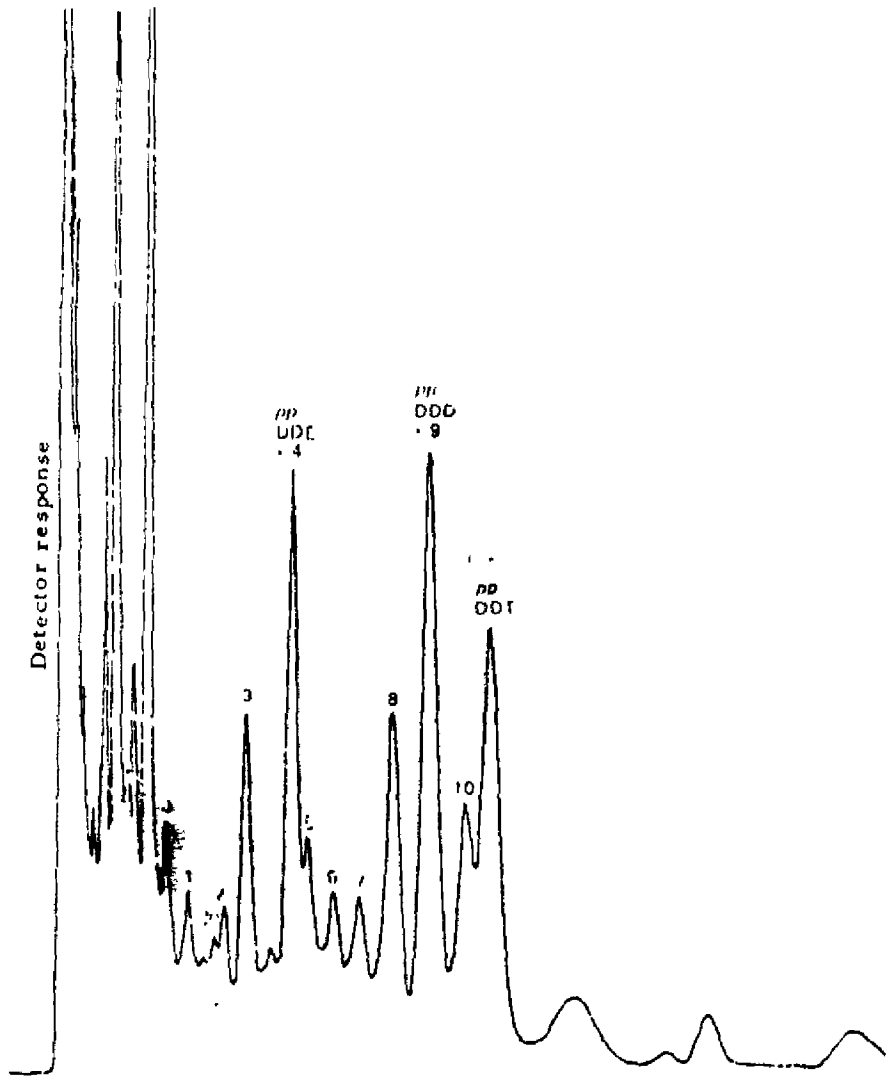
CHROMATOGRAM OF A MIXTURE OF PCBs
AND DDT AND ITS METABOLITES



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Fig 2(a)

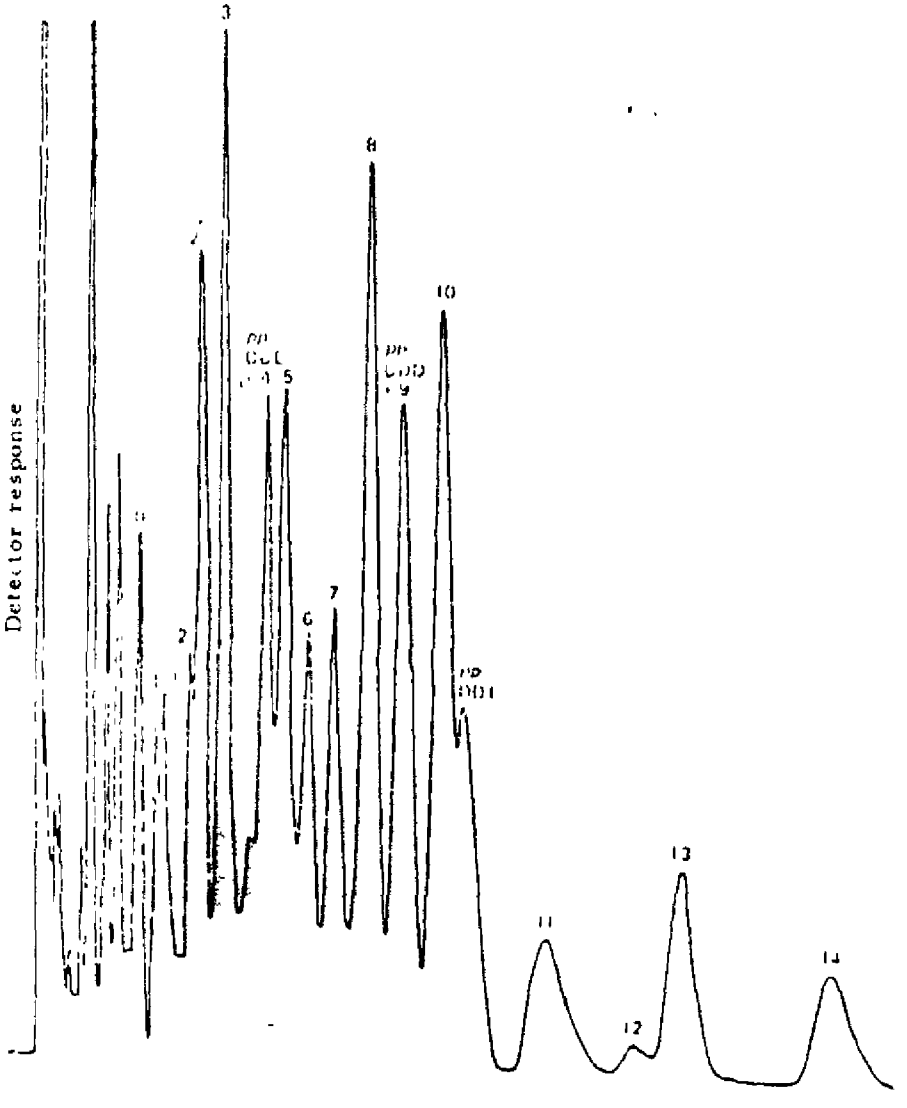
CHROMATOGRAM OF HERRING SAMPLE



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Fig 2(b)

CHROMATOGRAM OF PLANKTON SAMPLE

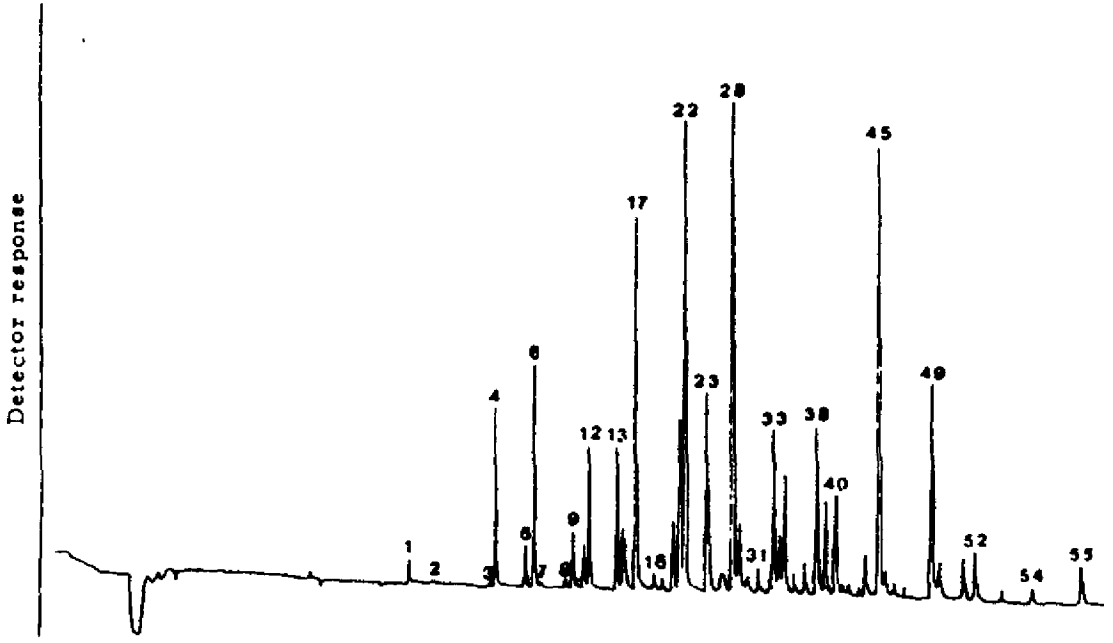


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Fig 3

CHROMATOGRAM OF CLOPHEN A60
(OBTAINED WITH ELECTRON-CAPTURE DETECTOR)

Column: Glass capillary, 60 m x 0.33 mm I D
Stationery phase: SE-30
Temperature: Programmed 180-260°C at 2°C/min



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2.4.1 Extraction

PCBs can be extracted by different methods that may vary from laboratory to laboratory; a summary of the methods reported in the literature for both animal tissues and aqueous samples has been given by Nelson (1972). While sediments and biological samples are normally extracted directly, extraction from water and air necessitates the use of relatively large samples. Pre-concentration by means of adsorbents, such as polyurethane foam, undecane-coated or silicone-bonded supports, or special resins, is slowly replacing the older liquid-liquid partition technique. With all techniques, there is a risk that the extraction may not be sufficiently effective. The conventional test of the efficiency of extraction, in which a known quantity of the substance under investigation is added to sample material, is not completely satisfactory because it is based on a post mortem addition of the substance. Extraction from the spiked sample is then assumed to be as effective as that from a natural biological sample, but this may not be true, as the former may be easier than the latter. Only if radioactively labelled test substances are introduced into the animal in vivo can a recovery experiment be regarded as satisfactory. Methods of analysing blood and milk using hexane as the extracting solvent have been reported in the literature. When a conventional recovery experiment is carried out, in which PCBs and DDT are added to milk or blood, the recovery is close to 100%. If, however, the hexane method is used on unspiked blood or milk, and an acetonitrile-formic acid mixture is used in addition, the recovery is increased 10-fold.

2.4.2 Clean-up

This step serves mainly to remove unwanted co-extracted materials, particularly lipids. Clean-up can be either destructive or non-destructive.

2.4.2.1 Destructive clean-up

Lipid extract in hexane can be treated in a closed test tube with either sulfuric acid containing 7% of sulfur dioxide or with alcoholic potassium hydroxide (Jensen, 1972). In both cases, PCBs remain unchanged while sulfur trioxide destroys dieldrin and heptachlor epoxide, and the alkali transforms DDT into DDE, and DDD into DDMU. The analysis of biological samples is, however, most often performed to provide data on the presence of a wide variety of contaminants. Destructive clean-up methods are therefore not always suitable.

2.4.2.2 Non-destructive clean-up

Two methods, adsorption chromatography and liquid-liquid partition, are in common use. In adsorption chromatography, chlorinated hydrocarbons are eluted from specially prepared alumina or Florisil columns with solvents less polar than lipids (Holden and Marsden, 1969, Porter and Burke, 1973). Numerous modifications have been worked out. When highly activated supports are used, large volumes of solvent are often necessary to elute the substances from the column. In some modifications,

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the adsorbent is deactivated by the addition of water to reduce the effluent volume required. One general disadvantage of adsorption chromatography is that the column is easily overloaded. This gives poor sepration between the fat and the chlorinated hydrocarbons, and thus less reproducible results For these reasons, many analysts use an additional clean-up step based on liquid-liquid partition between two immiscible liquids prior to column chromatography. One liquid is always hexane, while the other can be N, N-dimethylformamide, acetonitrile or dimethylsulfoxide. Both solvents are saturated with each other prior to partition. The chlorinated hydrocarbons are preferentially concentrated in the polar phase and the lipids in the hexane phase. After proper dilution of the polar phase with water, the organochlorine compounds are re-extracted with fresh hexane. After evaporation of most of the hexane, this solution is transferred to an adsorption column for final clean-up. When this procedure is used, a very clean solution free of fat is obtained.

A disadvantage of both methods is, however, that they are time consuming and that very large amounts of expensive solvent are used. Another disadvantage is that most solvents contain electron-capturing impurities; these can cause very serious problems if they are concentrated in a small final volume. Solvent purification by redistillation is therefore necessary.

A clean-up method involving liquid-liquid partition chromatography has recently been developed that seems to combine very good separation efficiency between fat and chlorinated hydrocarbons with the use of extremely small solvent volumes (Jensen and Sundström, 1974). Another clean-up method makes use of the gel permeation principle (Stalling et al , 1972).

2 4 3 Fractionation and derivatization

In the extreme case where PCBs alone are present in the sample, analysis consists only of treatment with sulfuric acid to destroy lipids followed by injection into the gas chromatograph. Usually, however, DDT and its main metabolites, DDD and DDE, are present in the sample together with PCBs. It can be seen from figs 1(c), 2(a) and 2(b) that DDT, DDD and DDE interfere with certain peaks for PCBs. PCBs can easily be separated from DDT and DDD by adsorption chromatography, using silica gel or activated carbon columns. The use of silica gel to fractionate organochlorine residues has been described, for example, by Holden and Marsden (1969), and Armour and Burke (1970). To separate DDE completely from PCBs with silica seems, however, to be almost impossible Many different methods have been described, but the separation reported is not good enough for use in a routine method (Masumoto, 1972) The solution to this problem may well be provided by the use of high-pressure liquid chromatography or the achievement of improved separation by gas chromatography.

However, methods exist whereby PCBs can be obtained free from DDE interference by treatment with chromic acid (Collins et al., 1972), this converts DDE to dichlorobenzophenone, which can be seprated from

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PCBs by either adsorption chromatography or directly in the gas chromatograph

Recently, Berg et al (1972) reported that they had been able to separate all PCBs from DDT, DDD and DDE (and also from many other chlorinated pesticides). These authors used a column filled with activated carbon for the separation, and found it possible to elute all DDT and DDT metabolites by means of a 3:1 mixture of ether and acetone PCBs were eluted with benzene. They investigated several activated carbons of different origin and found that the choice of charcoal was critical; only Fisher "Coconut carbon for decolorizing" was suitable. The recovery was about 90% for all substances, Jensen and Sundstrom (1974) found that PCBs, DDT and DDT metabolites were fractionated on activated carbon according to their chlorine substitution patterns. The order of elution from the column was: (1) o, p (DDD + DDT); (2) DDT; (3) DDE; (4) all PCBs with chlorine in the 2, 2'6, 6' position; (5) all PCBs with clorine in the 2', 2, 6' position; (6) all PCBs with clorine in the 2, 2' position; and (7) all PCBs with clorine in the 2 position only.

With a proper selection of the stationary phase, it is possible to separate by gas chromatography all the 4 ortho and 3 ortho compounds from each other and from interference by the DDT group. As some 1- and 2 ortho-polychlorinated biphenyls overlap with some of the 3- and 4-ortho compounds even on the most suitable conventional gas chromatography column, it would be an advantage for these substances to be present in different fractions.

2.4 4 Gas chromatography

No column coating designed for conventional analysis of chlorinated pesticides has so far made it possible to separate all the pesticides from PCBs, or to separate all the PCBs from one another. The stationary phase that gives the most effective separation of PCBs by gas chromatography appears to be Apiezon L. As an example, the peak marked number 10 on the column giving the chromatogram shown in fig. 1(a) will, on an Apiezon L column, be peak number 18 (fig. 4). It must, however, be remembered that even an Apiezon L column will not separate all major PCBs. The separation of PCBs on a conventional column using Apiezon L is improved by purification of the normal yellow Apiezon L grease by chromatography on alumina with hexane as the eluting solvent (Jensen and Sundstrom, 1974).

2.4 5 Quantitation

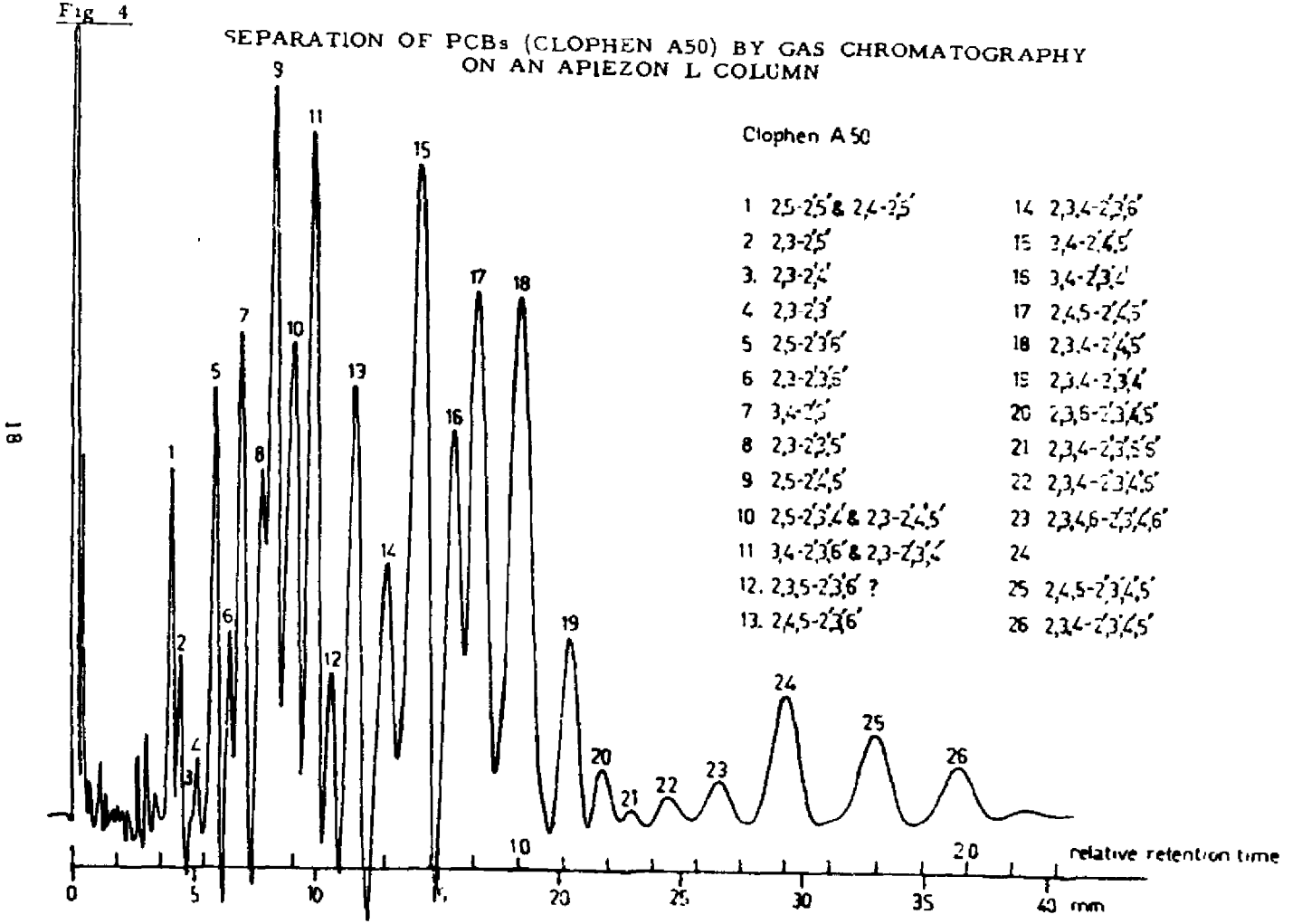
There is no generally accepted method for the quantitation of PCBs, but most techniques involve the use of the heights of areas of one or more peaks, recognized as PCBs For a summary of these techniques, see Nelson (1972).

The quantitation obtained by using conventional gas chromatography cannot possibly be accurate in respect of the content of individual PCBs,

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Fig 4

SEPARATION OF PCBs (CLOPHEN A50) BY GAS CHROMATOGRAPHY ON AN APIEZON L COLUMN



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but it can be reasonably precise for the estimation of total PCBs. This was demonstrated by the results obtained in an OECD international study (Holden, 1973)

Some of the methods used in practice are described below.

(a) Quantitation from the individual peaks of the chromatogram

In this method, a standard formulation is used and each component of the standard, separated on a gas chromatography, is quantitated individually. A commercial formulation that most closely resembles the pattern of PCBs found in biological material is chosen as the standard. This formulation is quantitated by using a mass spectrometer and a microcoulometer. With a mass spectrometer coupled to the gas chromatographic system, the number of chlorine atoms for the components in each peak can be determined. With the same gas chromatographic system attached to a microcoulometric detector, the amount of chlorine in each peak can be estimated. By combining the qualitative and quantitative information thus obtained, the concentration of PCBs in each peak in the standard can be determined (Jensen et al., 1969, Webb and McCall, 1973).

The peak height of each component in the standard is compared with the corresponding peak height in the sample, and the amount of each component calculated. Since the peaks do not represent single components but mixtures of PCBs, the method is not completely satisfactory Nevertheless, it gives some indication of the changes in the pattern of PCBs that take place in nature Good gas chromatographic separation is required. This implies that both support material and stationery phase must be handled carefully to give a high number of theoretical plates The procedure is rather time-consuming, as it requires measurement of all peaks.

(b) Quantitation on the assumption that amounts of PCBs can be estimated by using the response of DDE as a reference

In one version of this procedure, each individual peak height is multiplied by the corresponding retention time and all the products thus obtained are summed. This sum is then divided by the product of the peak height and retention time for pp'-DDE to give the total amount of PCBs (Collins et al., 1972).

The electron-capture response for PCBs with 4-9 atoms of chlorine per molecule (as normally found in biological samples) is reported not to exceed 1.6 times the value for pp'-DDE (Zitko et al., 1971) However, the response of each component relative to that of pp'-DDE is variable. Although this method is said to give reliable values for the total content of PCBs, the results must be erroneous when individual components are to be estimated.

(c) Quantitation based on an average of certain peaks when similarity to a certain formulation is assumed

In this method, the chromatographic pattern of the sample is studied, and the degree of resemblance to the various standard formulations is evaluated. Once a certain formulation has been adopted as standard, the height or area of a peak from the sample is compared to that of the corresponding peak in the standard, and the total content of PCBs calculated. If several peaks are considered, an average value is estimated. This method is rapid and ideal when the chromatogram of the sample is identical to that of the standard. It is, however, well known that the chromatograms of biological samples seldom resemble those of the standard formulations used. This may be explained by assuming that the samples contain a mixture of PCBs from different sources The fact that degradation of some of the PCBs occurs also reduces the reliability of the method. Selective accumulation of different PCBs also constitutes a source of error. The choice of representative peaks will be arbitrary. Attempts have also been made to quantify PCBs in terms of more than one commercial mixture (Stalling et al., 1972), but it must not be assumed that the residues found in the environment have been derived from those mixtures in the proportions found.

Although there are slight differences between the various commercial mixtures containing similar precentages of chlorine, these are small in comparison with the differences arising from other sources in the analytical technique.

(d) Quantitation based on the single peak obtained after perchlorination of PCBs

It has recently been reported that samples analysed for PCBs have, after clean-up, been treated with antimony pentachloride. Berg et al., 1972 Mizutani and Matsumoto, 1972). The components are thereby chlorinated to decachlorobiphenyl. The chromatogram, consisting of a single peak, is then compared with a similarly treated standard, or with a synthetic decachlorobiphenyl standard.

This method calls for effective clean-up and separation prior to gas chromatography. It demands a reproducible chlorination procedure, but the reported yields are stated to be around 85%

(e) Quantitation by means of synthetic mixtures

As can be seen from fig. 2, there is a shift in the pattern of PCBs in biological materials when compared to the formulation shown in fig. 1(a). To be able to understand the reasons for such shifts, there is a need to identify all the components present in the formulations This is best achieved by synthesizing all the more important PCBs When these substances are mixed in the right proportions, this mixture can be used in the same way as the standard pesticide mixtures normally used in gas chromatographic identification and quantitation

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Thanks to the excellent pioneering work done by Sissons and Welti (1971) in identifying most of the PCBs in the different formulations, great progress has been made. With the help of the information obtained, it has been possible to synthesize more than 95% by weight of the PCBs present in the formulations containing 50% and 60% chlorine (Jensen and Sundström, 1974). The PCBs are mixed in the same proportions as in the formulations and used for quantitation of PCBs in biological samples.

2.5 Determination of environmental concentrations of PCBs

We have here dealt with different methods for the processing of the sample and quantitation of PCBs. These can, of course, be combined in order to attain the highest possible efficiency in the determination. However, it is questionable whether the highest degree of accuracy is always necessary. An analytical method involving many stages will restrict the number of analyses that a laboratory can carry out. It may therefore be necessary to choose between analysing a few samples with the highest degree of accuracy or carrying out a large number of analyses with a lower degree of accuracy. The choice will naturally depend on the aim of the analyses.

As is well known, biological accumulation can vary a great deal from one animal to another, even under identical conditions. This variation is certainly greater in the natural environment, where animals live under widely differing conditions. It is therefore important, from a statistical point of view, for a sufficiently large number of analyses to be performed. This may mean that the highest degree of accuracy cannot be achieved for each sample, if the main aim is to establish total concentrations of PCBs within certain areas and at different ecological levels.

The quantitation of the PCBs should always give reproducible results, but if the values obtained are also to be correlated with those from other countries, it is very important that the comparability of the different methods used should be tested. Very often, reviewers compare levels of PCBs published in the literature, without considering the reliability of the different results.

Apart from the study of the levels of PCBs in different areas, the importance of the degradation products is now being increasingly realized. Until recently, it was thought that PCBs were almost undegradable, but it is now known that this is not the case. Furthermore, it has been found that individual PCBs behave differently. Some are easily degradable while others seem to be more persistent. In the future, it will be necessary to develop methods of analysis for degradation products or metabolites of PCBs. It is not known whether it is the PCBs present in samples or those that are degraded that are responsible for biological effects. The recent discovery of biologically active by-products in some formulations, such as chlorinated dibenzofurans (Vos et al., 1970), has also to be taken into consideration when analysing wildlife samples. In this type of study it is necessary therefore to determine all the individual

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components, while the need for the analysis of a large number of samples will be smaller. Fortunately, even when the levels of PCBs vary between individuals of the same species within a given area, the pattern is often quite similar. It has recently been reported (Maugh, 1973) that DDT can be degraded in the atmosphere by ultra-violet irradiation to yield certain PCBs. The likelihood of this process making any significant contributions to the total content of PCBs by the biosphere is remote, in view of the fact that those PCBs occurring most frequently contain five or more chlorine atoms, whereas the PCBs predicted by Moilanen and Crosby (Maugh, 1973) contain two to four chlorine atoms.

2.6 Recommendations

1. Routine techniques for the separation, identification and measurement of individual PCBs in environmental samples are urgently required and the use of capillary columns in gas chromatography should be developed. If this technique proves successful, it will be possible to improve the identification and to automate the measurement of both PCBs and other organochlorine compounds.

2. Standard mixtures of all commonly-occurring PCBs should be made available for use in the quantitation of environmental samples. PCBs should also be added to biological samples in all laboratories engaged on the analysis of these compounds.

3. Methods of analysis for residues of polychlorinated terphenyls, chlorinated naphthalenes and long-chain chlorinated paraffins should be developed, in view of their possible use as substitutes for PCBs.

4. Analytical methods are required for determining the metabolites and degradation products of PCBs in environmental samples.

5. Improved techniques are required for the sampling and extraction of PCBs in air, water and sediments, especially from relatively uncontaminated areas.

6. Commercial technical-grade mixtures of PCBs should be routinely analysed for chlorinated dibenzofurans. Similar analyses should be performed on such mixtures that have been used in heat-transfer systems and hydraulic transmissions.

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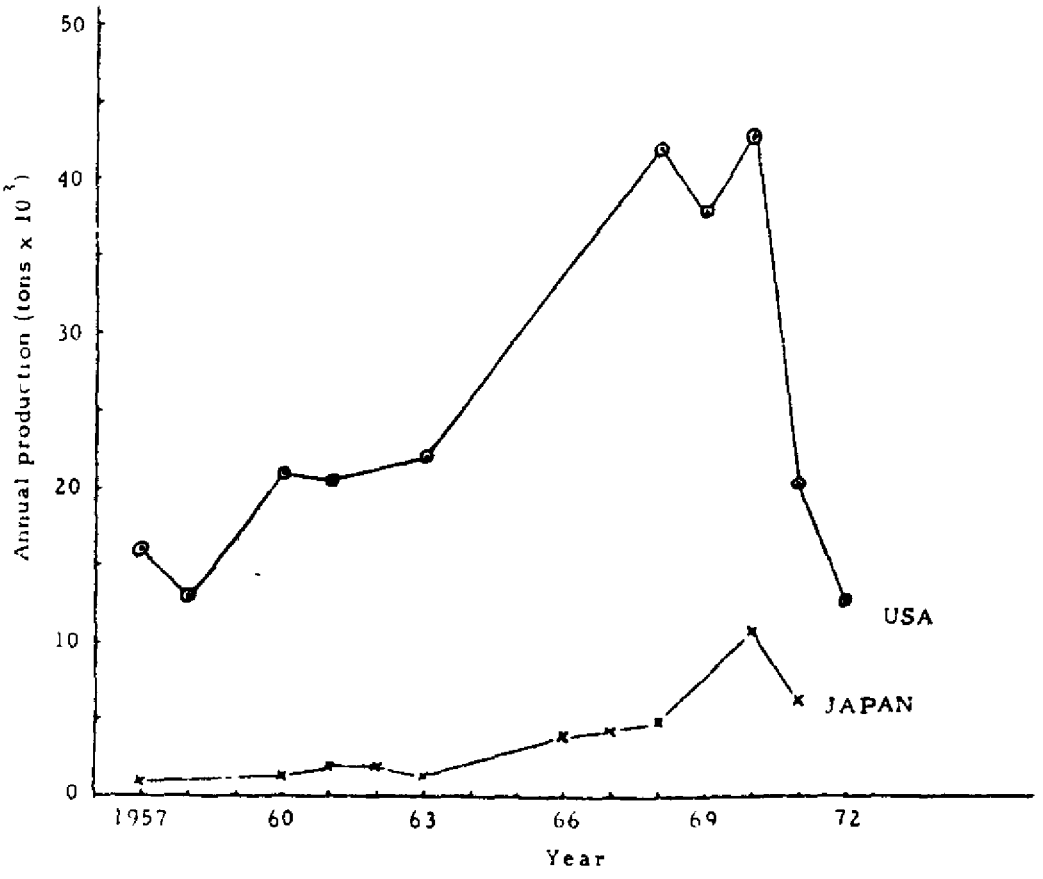
3 SOURCES OF ENVIRONMENTAL POLLUTION

3 1 Industrial production

During the last 15 years, about 500 000 tons of PCBs have been produced in the United States of America, while Japan has probably produced more than 130 000 tons. It can be estimated, therefore, that more than one million tons have been produced in the world since the 1930s (fig. 5 and table 3).

Fig 5

ANNUAL PRODUCTION OF PCBs IN THE USA AND JAPAN



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Table 3

PRODUCTION OF PCBs IN OECD COUNTRIES IN 1971

Country	Production (tons x 10^3)
United States of America	18
Federal Republic of Germany	8
France	7.6
United Kingdom	5
Japan	6 8
Italy	1.5
Spain	1.5
	Total 48.4

Although hundreds of specific products and applications are involved, these can be broken down into relatively few categories. Table 4 shows an OECD compilation of uses in various categories for a number of countries in 1971.

3 2 Utilization patterns

PCBs were never intended to be dispersed into the environment, as were DDT and other insecticides. However, with the advantage of hindsight, consideration of the physical and chemical properties, the uses tabulated in table 4, and knowledge of prevailing waste disposal practices, it could be predicted that universal contamination of the environment was inevitable. Transformers, heat exchangers, and hydraulic systems do leak sooner or later.

Plastics, paper, cardboard, used cutting oil and waxes are discarded in dumps, or are burned in inefficient fires or incinerators. Small capacitors, used in many domestic electrical appliances, may also represent an important source of contamination.

PCBs are used and escape into the environment almost exclusively on land, but can be leached out of the various products by natural waters and be transported by the major hydrological routes to estuaries, coastal environments, or merely to the next town. The fraction of the PCBs that is volatilized, either by fire or solar energy, can be transported to another continent via the major wind patterns of the troposphere. It has been suggested, and seems probable that, because of the very low vapour pressure of the molecules involved, entrainment of PCBs as droplets, or in the

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Table 4

CONSUMPTION AND USES OF PCBs IN OECD MEMBER COUNTRIES IN 1971

Countries	Consumption in 1971 (tons)	Uses (tons)								
		Dielectric fluids	Heat Exchangers	Hydraulic fluids	Cutting oils	Paints and varnish	Inks	Adhesives and plastics	Plastics	Resale
Federal Republic of Germany	6 150 [a]	3 600	30	1 000		-		1 500		
Belgium	1 350	1 290				60				-
Canada	2 180 [b]	2 100		-		-			-	-
Spain	1 500 [c]	-		-		-				
United States of America	17 000 [d]	13 000	1 600	-	800	-		1 600		
Finland	200	200				-	-			-
France	5 640 [e]	4 590		150	270	250	15	200	60	105
Japan	7 100 [f]	-		-	-	-		-	-	
Norway	35	10			-	10			15	-
Netherlands	400	-		-		-				-
Portugal	36 [g]	-		-		-			-	-
United Kingdom	1 100 [h]	1 100			-	-	-		-	-
Sweden	400	400	-	-		-		-		
Switzerland	480	-		-	-	-			-	-

[a] Of which 3 000 tons PCBs containing 42 % chlorine

[b] Of which 270 tons PCBs containing 42 % chlorine 1 360 tons PCBs containing 54% chlorine and 470 tons PCBs containing 60 % chlorine

[c] Estimated value

[d] Of which 725 tons PCBs containing 21 % chlorine, 9 500 tons PCBs containing 42 % chlorine 2 700 tons PCBs containing 54% chlorine and 800 tons PCBs containing 60 % chlorine

[e] Of which 314 tons were imported mixed with polychlorobenzenes

[f] Production plus imports

[g] The figure is for 1970

[h] Of which 800 tons PCBs containing 42 % chlorine, and 300 tons PCBs containing 54 % chlorine

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smoke of incinerators or of burning dumps, must be a major atmospheric vector (Ui, 1972, Jernelöv, 1972), this suggestion also helps to explain the tendency for environmental samples to exhibit only slightly distorted chromatographic peak patterns characteristic of specific commercial mixtures of PCBs.

4. ENVIRONMENTAL SOURCES OF EXPOSURE AND EXPOSURE LEVELS

As already pointed out, PCBs are used and escape into the environment almost exclusively on land. For this reason, more environmental data on local concentrations of PCBs in food and water supplies would be expected, since these are the areas of greatest concern from the point of view of health. However, these are also just the areas where fluctuations in the concentrations of PCBs are large, so that very little information on long-term trends is available. Conversely, concentrations of PCBs in those parts of the world remotest from the regions of major use, e.g., the water and the organisms of the deep sea, and the snows of the polar caps, furnish the most information on long-term trends and for predictions and baseline data for the world, but are of little value for local or regional health assessments. With few exceptions, there is far more information on localized conditions in meat, fish and poultry, etc. The major exceptions are birds of prey, seabirds, and marine mammals. The general interest among environmental scientists in such organisms, and the realization that they are more liable to be exposed to pollutants and to reflect the conditions existing in broad areas of the earth in their food intake have led to the collection of a great deal of information on their levels of PCBs.

The problems of the atmospheric distribution pathways for PCBs are emphasized because data (Harvey et al., 1972, 1974; Harvey and Steinhauer, 1974) substantially confirmed by Williams and Holden (1973) suggest that these must be chiefly responsible for the enormous amounts of PCBs now found in the oceans. Ui (1972) suggested that as much as 20 of the annual production of PCBs was released into the atmosphere, compared to only 5% released to land or inland waters. In contrast, Nisbet and Sarofim (1972) estimated that, of the amount produced in the United States of America in 1970, the equivalent of about 6% was released to the atmosphere and the equivalent of about 77% to land and fresh water.

Recent studies on the sensitivity of various species of marine plankto to environmental stress have shown appreciably higher resistance in those collected near the shore as compared with that of others from the open sea Moreover, it was found that species grown in two-species mixed cultures are more susceptible than those grown in single-species culture, perhaps because of competition effects (Fisher et al., 1973, Honjo et al. and Remsen et al., unpublished data). These findings give further support to

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the conclusion that any further increase in the environmental burden of PCBs may give rise to unexpected environmental hazards.

4.1 Exposure to the general population

In air remote from immediate sources of waste discharge, levels of PCBs are only a few micrograms per cubic metre (Interdepartmental Task Force on PCBs, 1972). The major components of air-borne PCBs are the more volatile, low chlorinated homologues that are readily excreted from the body and are present in very low concentrations. The inhalation of PCBs therefore makes a very minor contribution to human intake in non-occupational exposure.

Dermal absorption by contact with re-used paper or non-carbon copying paper or plastics containing PCBs would be possible. Washing the hands with soap removes only approximately 60% of the PCBs (Kuratsune and Masuda, 1972).

The major route of intake of PCBs is the oral route, to which drinking-water makes a very small contribution. In water remote from sources of water discharge, levels of PCBs are less than 1 µg/kg (Interdepartmental Task Force on PCBs, 1972).

Many kinds of food have been contaminated with PCBs either by accident or by contact with materials containing PCBs. Apart from such sporadic instances of contamination, PCBs have been detected in fish and marine mammals (whales and seals) at considerable concentrations. Other food items, such as milk, meat, eggs, cereals and vegetables, never attain such high levels under usual circumstances (Environ. Res., 1972).

The quantity of fish consumed varies with traditional diet and habits or individual food choice in each country, as well as with the type of occupation (fishermen, fish market employees, etc.). Levels of PCBs in fish vary over a wide range depending on the degree of contamination of the water. Most ocean fish have levels in the range 0-0.5 mg/kg with the exception of certain species with levels up to 2 mg/kg. Inshore fish or fresh-water food fish have much higher levels of up to 10-20 mg/kg, depending on local conditions (Environ. Res., 1972).

The contamination of human milk with PCBs has given rise to some concern as it may lead to the intake of these compounds by babies.

Milk from cows on farms in the United States of America where the silos were contaminated with PCBs contained 1-10 mg/kg PCBs in the fat. Samples from Sweden had much lower levels (Berglund, 1972), averaging less than 0.5 mg/kg in the fat over the period 1967-1969. Kolbye's (1972) data show an average concentration of 2.27 mg/kg in the fat of a number of positive samples of a total sample of 941 commercial cows' milk samples. The mean level in whole human milk from two cities in California was about 60 µg/kg (Risebrough and Brodine, 1969). It was 16 µg/kg in Sweden

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or 0 5 µg/kg on a fat basis (Westoo et al., 1970), and 100 µg/kg in the Federal Republic of Germany, or 3.5 µg/kg on a fat basis, (Acker and Schulte, 1970). In Japan, the mean level of PCBs in whole human milk was 36 µg/kg but in breast milk of mothers living around the contaminated inland sea, it was 60 µg/kg, reflecting the higher degree of contamination of fish in this partly enclosed narrow sea. If it is assumed that the daily milk intake is 150 g/kg, breast-fed infants in California and some districts of Japan would ingest around 9 µg/kg day of PCBs. In the case of the Federal Republic of Germany, the calculated figure would be 15 µg/kg day An intake of this magnitude is far greater than the mean daily intake (MDI) for adults. Moreover, it is reasonable to assume that sensitivity to PCBs is higher in infants than in adults. Kuratsune et al. (1972) calculated that the minimum daily intake that produced clinical symptoms of Yusho disease in adults was 70 µg/kg over a period of four months. This is only five times greater than the calculated value of 15 µg/kg day consumed by babies as noted above.

4.2 Occupational exposure

Meighs et al (1954) reported cases of chloracne among workers exposed to Arochlor for 19 months. The concentration of Arochlor in the air at the work-place was estimated to be 0.1 mg/m^3. Hara (1969) also reported similar occupational cases of chloracne caused by prolonged exposure to Kanechlor and/or Arochlor.

4.3 Estimate of effective human exposure from all environmental media (daily intake)

Estimation of the mean daily intake (MDI) is rather difficult; if the average level of PCBs in fish is used in the calculation, this will lead to an over-estimate.

In the United States of America, the mean value of the ratio Σ DDT/PCBs for market-basket samples appears to be around 4 (deduced from the data of Kolbye (1972) and Corneliussen (1970). Since the MDI for Σ DDT is about 40 µg/day (Duggan, 1969), the MDI for PCBs can be calculated as round 10 µg/day.

In Japan, the average daily intake of fish was estimated as 86.3 g from the national nutrition survey of 1971. Taking the average level of PCBs in fish as 0.5 mg/kg, the MDI for adults will be 47.7 µg/day on the average, the values ranging from 40.3 to 72.4 µg/day. However, analysis of a whole day's diet per person in Kyoto city gave the MDI as 21 µg/day. Calculation from statistical data tends to result in over-estimation.

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5. ECOLOGICAL EFFECTS

5.1 Levels in soils and terrestrial organisms

There do not appear to be any published data on PCBs in soils. However, McClure and LaGrange (1973, unpublished data) found on the surface of 12 soil cores taken at La Jolla, California, an average concentration of PCBs that was five times greater than the average concentration they found at a depth of 10 cm in the same cores. Evidently PCBs had reached the soil surface and moved downwards by some process not now understood. More such studies are clearly needed for agricultural soils of many types

PCBs are widely distributed and are probably universally present in terrestrial ecosystems in both North America and Europe. The highest concentrations within a food web are generally found in raptorial birds that prey largely or in part on other birds; North American examples include the peregrin falcon (Falco peregrinus), prairie falcon (F. mexicanus) and merlin (F. columbarius), breeding on the prairies of Western Canada, and the gyrfalcon (F. rusticolus), breeding on the tundra High levels of PCBs have also been detected in birds of prey and owls in various European countries, including the United Kingdom, the Netherlands and Sweden (Prestt et al., 1970; Fuchs et al., 1972; Odsjö, 1973) Levels of up to 100-500 mg/kg on a fresh tissue basis have been observed, for instance, in the livers and pectoral muscles of long-eared owls (Asio otus) and eagle owls (Bubo bubo) Other species of birds feeding on foods such as insects and seeds are also contaminated with PCBs, but at lower levels (Canadian Wildlife Service, Ottawa, personal communication)

The effectiveness of terrestrial food chains as pathways in the transfer of PCBs to man is by no means fully clear. In a study by Hammer et al. (1972), PCBs were found in the plasma of 81% of refuse workers but in that of only 11% of the controls This observation strongly suggests the existence of at least one non-food chain pathway to man.

5.2 Levels in fresh-water and associated organisms

Since the sources and uses of PCBs are located almost exclusively on land, much higher levels of PCBs in nearby fresh-water systems would be expected than in the oceans. This is indeed true for fresh-waters near sources of PCBs, but fish in some rivers and lakes remote from industry have concentrations of PCBs lower than those reported in the open North Atlantic Ocean. This can be attributed partly to the localized pattern of the major wind systems responsible for the atmospheric transport of PCBs - counterbalanced in the oceans by the horizontal circulation systems associated with them - and partly to the relatively rapid rate at which the surface waters of fresh-water systems, and even large lakes, are exchanged and groundwaters cleansed by contact with soil, or purged by the scavenging of PCBs by sinking particles.

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Concentrations of PCBs in the water of the Milwaukee River, a typical example of a river used for industrial purposes, are in the range 4-250 μg/kg (Veith and Lee, 1971). The fish in the river had concentrations between 2 and 400 mg/kg. Among the 14 species examined, no consistent trend in either concentration or in mixtures of PCBs was found (Veith and Lee, 1971). The levels in water and fish reflected the numerous localized discharges of PCBs into the river. The fact that there was no correlation between the concentration of PCBs in the fish and their size or lipid content cannot be used as evidence for lack of food-chain magnification, since sporadic proximity to discharges will cause temporal fluctuations in concentration A fresh-water ecosystem in a heavily industrialized region of southern Sweden was studied at monthly intervals for its content of DDT and PCBs. No simple relationship was found between the concentrations of PCBs in nine components (including crustaceans, insect larvae, molluscs and salmonid fish) of the ecosystem (values ranged from 1 to 15 μg/kg on a wet weight basis) and their presumed diet. The authors concluded that the components of this particular ecosystem did not seem to store chlorinated hydrocarbons in a simple way (Sodergren et al., 1972).

Fresh-water systems in Japan have generally had very high concentrations of PCBs. Levels of between 10 and 20 mg/kg (wet weight) have been found in carp (Isono, 1972, personal communication).

Concentrations of PCBs in sewage sludge from Glasgow, Scotland, ranged from 0.1 to 14 mg/kg (wet weight), equivalent to a discharge of 1 ton/yr (Holden, 1970). The PCBs discharged in this way rapidly reached coastal waters, from where they entered the ocean (Williams and Holden, 1973).

5 3 Levels in the oceans and in marine organisms: sampling problems

The collection, at sea, of samples to be analyzed for PCBs at the very low concentrations expected (and often found) in open ocean waters and plankton organisms gives rise to a series of problems of great difficulty. Grice et al. (1972) and Jensen et al. (1972) have drawn attention to the extensive use of PCBs in marine paints, in the hydraulic fluids of much sea-going gear, in engine-room wastes, and so on. Grice et al. (1972) offered some suggestions for avoiding the contamination of samples from these sources, but were unable to prove that the suggested procedures were generally successful.

In interpreting the report of Jensen et al. (1972), it should be borne in mind (authors' erratum slip) that their vessel was freshly painted no more than two months before each collecting trip; this would be quite unheard of with a larger vessel, 12-24 months being the more usual interval between bottom paintings. It thus appears that this report defines a worst-case situation. Grice et al. (1972) and Harvey et al (1972, 1973) asserted that no paints, hydraulic fluids, or engine room supplies containing PCBs were used on their research ships, but have not published supporting analytical data.

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Harvey and Teal (1973) offered evidence that they interpreted as meaning that net plankton samples may extract PCBs from nets that have previously extracted these substances from the water. These authors and others (Williams and Holden, 1973) have also called attention to the problems posed by the inclusion in plankton samples of "tar balls" rich in PCBs. Since plankton nets are open to (and hence efficient collectors of) any ship-originating contamination both at the beginning and at the end of a tow, net plankton samples can be viewed as those most likely, and most seriously, to reflect contamination from these sources. As noted by Williams and Holden, (1973), "exceptionally high values must be treated with caution"

Water samples are usually collected with a device that is retrieved after being closed, it should thus be relatively immune from contamination after collection of the sample. In most cases, however, because of the pressure effects on an unfilled container lowered to any depth, water samplers are introduced into the sea while opened, and so are subject to contamination by the surface film or by the upper layers of water, both of which would be expected to be richer in PCBs than the water below them. It is usually hoped that such a source of contamination may be avoided by allowing the sampler to "flush" with deeper water for a period of time before it is closed, for some purposes, it has proved useful to lower the opened sampler to a considerable depth (1000 or 2000 m) for flushing, and bringing it back up to the sampling depth. Although tests of the efficiency of these procedures for PCBs have been begun, using samplers contaminated on the ship's deck by allowing solutions of 14C-labelled, highly insoluble organic compounds to evaporate within them (Harvey, personal communication), there are still too few data for a final evaluation to be possible. Experiments that have been performed (Harvey and Bowen, unpublished data) in which the concentrations of PCBs in samples collected with a stainless steel bucket (very effective for collecting the surface film) were compared with those in samples simultaneously collected by tripping the sampler of Bodman & Slabaugh (1961) just below the surface, showed consistently that the concentrations were about three times higher in the bucket samples, this evidently shows only that the bucket is a poorer way of collecting samples than the sampler of Bodman & Slabaugh. A method available for shallow depth samplee is that of introducing a stoppered, pre-cleaned bottle to the sampling depth, opening it in place and then restoppering it after it has filled. Holden reports (unpublished data) that samples obtained in this way showed lower mean levels of PCBs than those reported in the literature. No comparative studies in which this procedure has been used to test the performance of "lowered-open" samplers, like that of Bodman & Slabaugh, appear to have been carried out; such tests would be valuable, since the "closed-sampler" procedures are not usually practicable at depths beyond a few tens of metres.

Tests of this sort might well be used to demonstrate the ability of general-purpose (or all-depth) water-sampling devices to collect samples that are non-contaminated with PCBs from the collecting vessel or from the surface film. No comparable tests that could be used to confirm the freedom from contamination of plankton samples seem to have been

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performed It is certainly true that no published data on levels of PCBs in either sea-water or plankton, have been accompanied by convincing proofs of their validity.

5 3.1 Sea-water

Reported values are available only for the North Atlantic Ocean (Harvey et al., 1973). Surface-water concentrations between Bermuda and the Norwegian Sea in 1972 ranged from <1 to 150 ng/kg, the mean of 36 samples being 35 ng/kg. The mean of 11 samples from 200 m was about 10 ng/kg. No gradient was observed with changes in longitude, although slightly lower average concentrations were observed in the Sargasso Sea north of Bermuda. It must be noted (see the preceeding discussion) that these surface samples were collected by bucket, and those from deeper water with the sampler of Bodman & Slabaugh (1961); the difference between surface concentrations and those at 200 m is thus almost exactly that to be expected from the greater degree of inclusion of surface film in the bucket samples.

In view of the foregoing, and the other risks of contamination discussed above, it would seem unwise to use these mean concentrations for any elaborate mass balance calculations. However, the authors (Harvey et al., 1973) used their data to estimate a total content of PCBs of 20 000 tons in the mixed layer of the Atlantic Ocean. As they pointed out, this compared very favourably with the estimate of Nisbet and Sarofim (1972) that the North Atlantic could have received some 15 000 tons of PCBs up to the end of 1970, from the United States of America alone.

5 3 2 Plankton

Analyses of marine plankton have been reported by several groups (Harvey et al., 1972, 1974; Jensen et al., 1972; Williams and Holden, 1973). The data of Harvey et al. show much the greater spread of concentrations, as well as much the highest mean value. The samples of Jensen et al. from the Baltic, and of Williams and Holden from the North-eastern Atlantic, show almost identical ranges and very similar mean values. A disturbing aspect of the data of Harvey et al. is that no systematic difference, either in range or mean, was found between North and South Atlantic samples, even though, as the authors pointed out, the South Atlantic should have experienced much less contamination with PCBs

In view of the various discrepancies within and between these bodies of data, the fact that Jensen et al. showed that paint contamination had affected their samples, and the unsolved problems of preventing contamination in plankton sampling outlined above, the plankton data cannot usefully be interpreted. This is most unfortunate, because such samples, distributed among the first three stages (producers, herbivores, and first-stage predators) of the marine food webs, would clearly have much to tell us about the movements of PCBs both within ocean waters and in the marine biota

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5 4 Interaction effects

Great difficulties are met in trying to demonstrate a direct cause-effect relationship between the levels of any single contaminant (including PCBs) and effects observed in organisms This is a consequence of the fact that most effects are caused by the combined action of a multitude of physical and biological factors on the one hand and a large number of chemical contaminants on the other Organisms that contain high concentrations of PCBs frequently also have high levels of DDT, dieldrin, mercury, etc

Some interactions between contaminants can be explained on the basis of heptic microsomal enzyme induction by PCBs. Thus Ito et al. (1973, personal communication) recently found the feeding carcinogenic doses of butter yellow together with PCBs did not produce the anticipated incidence of liver tumours, no tumours were, in fact, seen. As the explanation, increased detoxification of the carcinogen was suggested

Enhanced heptic microsomal enzyme activity due to PCBs has also been put forward to explain the increased toxicity of carbon tetrachloride administered with PCBs (Grant et al., 1971b).

Ducks fed low levels of PCBs for 10 days and challenged the next day with a dose of duck hepatitis virus showed a higher mortality than ducks not pretreated with PCBs Even the low dose of 25 mg/kg of PCBs gave the same effect as one of 100 mg/kg.

Ito et al. (1973, personal communication) described a synergistic effect in carcinogenesis on feeding 100 mg/kg alpha-BHC (hexachlorocyclohexane) together with 250 mg/kg PCBs (Kanechlor 500). They observed hepatomas in some of the animals after 24 weeks In parallel experiments, in which only one or other of the chemicals were fed at the respective dose level, no hepatomas were seen in any of the animals.

A synergistic effect between PCBs and insecticides was observed by Lichtenstein et al. (1969) in Drosophila melanogaster and Musca domestica. No appreciable toxicity was observed after 48 hours' exposure to the PCBs Arochlor 1221, 1232, 1242, 1248 or 1254, all at the dose of 200 µg applied to the glass walls of the dry container for D. melanogaster or of 10 µg applied topically to the housefly. With pp'-DDT, an increase in mortality was observed for all PCBs tested on the housefly and for most PCBs tested on D melanogaster. The toxicity (mortality) was also considerably enhanced for both species with dieldrin as the insecticide, but not equally so for all the PCBs tested. In contrast, more highly chlorinated PCBs applied in the same fashion together with dieldrin or pp'-DDT decreased the toxicity or abolished it all together

At a non-toxic dose level (10 µg/fly), Aroclor 1248 increased the toxicity of the oxygen analogues of five organophosphate insecticides, but decreased the toxicity of parathion (Fuhremann and Lichtenstein, 1972).

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It is impossible to fit all those data together and to explain the mechanism of action involved in these interactions, particularly since the different samples of PCBs contained different components and possibly even different impurities. However, they clearly demonstrate the need to evaluate these and other interactions only after individual pure chlorinated biphenyls have been synthesized and tested in these systems.

5.5 Bioconcentration

Although the acute toxicity of PCBs is comparatively low, occasional individuals found dead of species of fish-eating birds had accumulated levels of PCBs that produce death under experimental conditions. Cormorants (Phalacrocorax carbo) found dead in the Netherlands have contained concentrations comparable to those shown to be toxic to captive specimens (Koeman et al., 1973). The source of the PCBs is assumed to be the fish in the estuary of the River Rhine upon which the birds feed.

Burdens of PCBs in guillemots (Uria aalge) found dead during the massive wreck of this species in the Irish Sea in 1969 have been calculated to be approximately twice that of healthy birds shot in the vicinity. Thus it is possible that PCBs contributed to the stress to which the guillemots had been exposed and were thereby implicated in the mortality (Parslow and Jefferies, 1973). Although data that might suggest that PCBs contribute to the mortality of other species are not at the present time available, mobilization of lipid during times of low food supply or of bad weather can be expected to increase the amounts of PCBs and of other fat-soluble pollutants in the body circulation and thereby to increase the internal stress.

Experimental studies with a variety of organisms have shown that PCBs may influence many biochemical reactions and physiological events. Other environmental effects of PCBs may therefore be found as additional research is undertaken; moreover, an increase in the environmental burden may influence sensitive species in ways as yet unsuspected.

It is generally believed that the storage of persistent compounds in animals is primarily dependent on their position in the food web. According to this model, the tissue concentrations are likely to be high in organisms at the end of food chains and low in those at the beginning. However, when the data now available on concentrations of PCBs and other persistent chemicals in various animal species are considered, it appears that, particularly in gilled and skin-breathing aquatic organisms, the concentrations found are not predictable from the trophic levels assigned to the organisms.

Thus the totality of available data does not support food-chain magnification of chlorinated hydrocarbons among fishes and other gilled organisms. Certain predators show levels of PCBs consistently higher than those of their prey, but the converse is true of others. Certain groups, such as sharks, plankton, cod and tunicates, have consistently higher levels of PCBs than do other symbionts, while trigger fish, barracuda, salps and shrimp always have lower levels Similar relationships have been

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observed with chlorinated hydrocarbon pesticides among fresh-water fish (Henderson et al., 1971). The data suggest that the concentrations of PCBs, or of any other chlorinated hydrocarbon, found in such aquatic organisms, are controlled by the concentrations in the water. The degree of partitioning of the PCBs across permeable body membranes into the lipid compartments is governed by the solvent quality of the various lipids. Thus, shark livers generally contain over 1 mg/kg of PCBs, while tuna and barracuda livers contain two orders of magnitude less. Chemical analysis of the lipid composition of the livers of these three species should reveal significant differences. It is also probable that the steric effects described by Jensen and Sundström (1974) must play an important role, especially in affecting the movement of isomers of PCBs into the lipoproteins of cell membranes, the first stage in uptake.

In air-breathing marine organisms, such as seals, whales and birds, the concentrations are controlled mainly by (1) the interspecies relationships, as far as the selection of prey species is concerned, and (2) the rate of retention of the compounds, as governed by the processes of absorption, biotransformation and excretion. Food-chain magnification may then occur in predators specialized in feeding on certain species of prey animals, where the latter are contaminated with a persistent pollutant.

Table 5 summarizes the mean concentrations of PCBs in several large groups of marine organisms, based on analyses of specimens collected over the last few years in the North Atlantic. The data on levels of PCBs found in a number of species of North Atlantic seabirds are summarized in table 6. The data show that concentrations may vary appreciably between species that share to some extent the same trophic level within the ecosystems concerned.

Table 5

AVERAGE CONCENTRATIONS OF PCBs IN VARIOUS CLASSES OF MARINE ORGANISMS FROM THE NORTH ATLANTIC

Organism	Concentration of PCBs[a] (µg/kg) (wet weight basis)
Mammals (muscle)	3 000
Seabirds (muscle)	1 200
Plankton	200[b]
Pelagic fish (whole body)	50
Mesopelagic fish (whole body)	10
Benthic invertebrates (whole body)	1

[a] Averages of data from various sources.

[b] See discussion on p. 30 of the uncertainties in the available data on plankton.

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Table 6

PCBs IN NORTH ATLANTIC SEABIRDS[a]

Species	Concentration of PCBs (mg/kg)[b] (wet weight basis)
Fulmar	4.
Great shearwater	0.1
Manx shearwater	0.3
British storm-petrel	8
Gannet	6
Great Skua	17
Glaucous gull	24
Lesser black-backed gull	6
Kittiwake	3
Guillemot	0.7
Puffin	0.2

[a] From Bourne and Bogan (1973)

[b] Average concentration in muscle

6 PHYSIOLOGICAL AND BIOCHEMICAL FACTORS RELATING TO MAMMALIAN UPTAKE AND STORAGE

6.1 Uptake and distribution

6.1.1 Absorption

Synthetic isomers of PCBs having from one to six chlorine atoms per molecule were fed to rats at doses between 5, 50 and 100 mg/kg body weight (Albro and Fishbein, 1970). The PCBs were very well absorbed: the retentions (fed minus excreted) of the various isomers were all greater than 90% of the amount fed regardless of the chlorine content.

6.1.2 Distribution

Grant et al. (1971b) administered 500 mg/kg of Aroclor 1254 orally to rats and analysed its distribution in tissues on the 4th and 24th days.

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The greatest concentration was found in the fat, and this concentration fell only by 33% in 20 days In other organs, the decrease in concentration over the same period was relatively rapid (64-90%). Aroclor 1254 was found to potentiate the toxicity of carbon tetrachloride The gas chromatographic pattern of the residues showed the increased contribution of the higher chlorinated components in the later stages (22nd day)

Curley et al. (1971) also studied the distribution and storage of PCBs in rats When Aroclor 1254 was fed at the level of 100 mg/kg, the levels of PCBs in fat did not reach a plateau even after 240 days while, in DDT feeding experiments, such a plateau was reached within 90-140 days

6 2 Target organ

The primary target organ of PCBs appears to be the liver. Marked liver necrosis and skin lesions were observed in rabbits after dermal exposure to PCBs (von Wedel et al , 1943). In a comparative dermal toxicity study in rabbits with Arochlor 1260, Phenochlor DP6 and Clophen A60, liver damage as well as acnegenic skin lesions were most severe in animals receiving those samples of PCBs that contained chlorinated dibensofuran impurities (Vos and Beems, 1971).

6 3 Body burden and critical organ burden levels in body tissues and fluids of humans poisoned by PCBs

The concentration of Kanechlor 400 in the subcutaneous adipose tissues of a Yusho patient was first estimated by Goto and Higuchi (1969) from the chlorine concentration to be 75 5 mg/kg at the face and 13 1 mg/kg at the abdomen. Kikuchi et al. (1971) reported the distribution of PCBs in the tissues of two autopsy cases of Yusho who died about a year after the onset of the skin symptoms. Taking the concentration of PCBs in the liver as 1 0, the relative concentrations in one of the cases were 9, 2, 9 0, 4 5, 7. 5, 3. 3, 5. 5 and 0. 2 for sternal marrow, right atrium of heart, right ventricle of heart, mesenterial fatty tissue, skin, trachea and brain, respectively. In another case, PCBs were found in sternal marrow and mesenterial fatty tissue, and in some other tissues, but not in brain. Kikuchi (1972) also reported an additional case of Yusho with liver cirrhosis and liver-cell cancer in autopsy in which a very small amount of PCBs was detected only in mesenterial and subcutaneous fatty tissues about two years after poisoning. Kojima (1971) found PCBs in the sputum of Yusho patients before May 1970 but rarely in samples collected after June 1970. Masuda et al. (1973) also analysed the liver and adipose tissues of Yusho patients for PCBs and found that the concentrations in the adipose tissues were slightly higher than the corresponding figures for persons not suffering from Yusho, as shown in table 7. In recent years, however, the levels of PCBs in Yusho patients have approached those in the general population.

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Table 7

PCBs IN TISSUES OF YUSHO PATIENTS AND OF PERSONS NOT SUFFERING FROM YUSHO

Subject	Time of death and of operation	Number of samples	Concentration of PCBs (mg/kg) (fat basis)[a]	
			Liver	Adipose tissue
Yusho Case 1	July 1969	-	0.1 (9.5)	1.3 (3.7)
Yusho Case 2	July 1969	-	0 2 (10.4)	2.8 (15.1)
Yusho Case 3	Nov. 1969	-	0.07 (1.3)	0.7 (8.4)
Yusho Case 4	Dec. 1970	-	0.07 (1.3)	1.7 (0.9)
Yusho Case 5	May 1972	-	0.08 (8 4)	4.3 (6.5)
Yusho Case 6	Sept. 1972	-	- -	1.9 (2.9)
Average	-	-	0.1 (6 5)	2.5 (6.3)
Control Average	Sept Nov. 1970	11	- -	0.9 (2.6)

[a] It was assumed that the PCBs consisted of a 1:1 mixture of Kanechlor 500 and Kanechlor 600.

6.4 Elimination, including transplacental excretion; biological half-life

The distribution and excretion of radioactive 2, 4, 5, 2', 5'-pentachlorobiphenyl (^{14}C-PCB) in mice was reported by Berlin et al. (1972). The isomer, after i.v. injection, quickly moved from blood to tissues after 20 minutes. The radioactivity of brown fat and liver were the highest on whole-body autoradiogram. After one day, fatty tissue was most highly radioactive, followed by the intestinal contents. The gall bladder and the bile were also active, but there was very slight penetration into foetal tissues. After 35 days, the most clearly defined areas were the lungs and the renal cortex. The excretion of radioactivity was fairly rapid, with a half-life of 6 days. The excretion pattern showed a two-phase excretion: the initial rapid loss corresponded to excretion from the liver, and the later linear portion to release from fat. Extrapolation of the falling curve showed that a reduction of the total body burden to 1% of its original value would require 65 days. A study with labelled 2, 4, 2', 4'-tetrachlorophenyl in mice and quail has also been reported (Melvas and Brandt, 1972). No major differences between the metabolism of this compound and 2, 4, 5, 2', 5'-pentachlorobiphenyl were observed.

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Yoshimura et al. (1971) administered tritium-labelled Kanechlor 400 orally to rats at a single dose of 20 mg/rat, and measured the body distribution and excretion of radioactivity after 3, 28 and 56 days. The highest concentration was found in skin and adipose tissue, but after 56 days the residue in the latter was several times greater than that in the former. Recovery from urine was only 2% of the amount administered, while 67% was found in the faeces.

6.5 The metabolism of PCBs

Klein (1973) injected (i.v.) 2,4'-dichlorobiphenyl-^{14}C and 2,2',5-trichlorobiphenyl-^{14}C into rhesus monkeys in doses of between 16.8 and 566 ug/kg of body weight. The biological half-life was 1.1-2.9 days for dichlorobiphenyl. After application of 2,2',5-trichlorobiphenyl, 82% of the radioactivity was recovered in the excreta after 14 days. The half-life was 2.1 days. The excreted radioactivity consisted only of a mixture of hydroxylated compounds, partly in conjugated form. In the case of trichlorobiphenyl, three monohydroxy products and one trihydroxy product were identified.

Yoshimura and Yamamoto (1973) studied the metabolic pathway of 3,4,3',4'-tetrachlorobiphenyl (TCB) in rats. They recovered three metabolites and unchanged (unabsorbed) 3,4,3',4'-TCB. It was found as the free form in the faeces; however, the possibility cannot be excluded that it was hydrolysed in the faeces from the glucuronide conjugates. Some metabolites showed increased toxicity, as compared to the original chlorobiphenyl.

7. TOXICOLOGICAL EFFECTS

7.1 Acute toxicity

The toxicity of PCBs has been the major cause of concern about their presence in the environment; this was originally estimated by analogy with the toxicity of DDT. The available evidence shows that, like DDT, PCBs do not possess a high acute lethal toxicity. As with the chlorinated pesticides, the dangers of PCBs arise from sub-acute and chronic doses and from their biochemical effects (especially on hormone balance).

The oral (LD_{50}) for albino rats is around several grams per kilogram of body weight. The highly chlorinated biphenyls, such as tetrachlorobiphenyl or higher homologues, are slightly less toxic (LD_{50} of 10 g/kg body weight) than trichlorobiphenyl (LD_{50} of 5-6 g/kg body weight).

The major components of residues of PCBs in the human body are penta- and hexachlorobiphenyl, and the chronic toxicity of these compounds

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is the key problem in the evaluation of health effects. The acute toxicity of PCBs is of little importance from the point of view of this report.

7.2 Sub-acute and chronic studies

7.2.1 Morphological changes, species comparison

Dermal application of 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl in rabbits produced marked liver damage, but the skin lesions were less severe than those produced by Aroclor 1260 (Vos and Notenboom-Ram, 1972). No differences in skin lesions were found when 3, 4, 3', 4'-tetrachlorobiphenyl and Kanechlor 400 were applied on the ear skin of rabbits (Komatsu and Kikuchi, 1972). Vos (1972) showed that liver effects are less marked in rats and monkeys than in rabbits; they consisted mainly of liver cell hypertrophy. Dermal application of PCBs to guinea-pigs had minimal effect on the skin (Miller, 1944). Hepatic porphyria has been found in chickens, quail, rabbits and rats (Vos, 1972), but not in guinea-pigs that died from exposure to PCBs (Vos and van Driel-Grootenhuis, 1972). In chickens, a remarkable finding was hydropericardium, subcutaneous and abdominal oedema, as well as tubular dilation in kidneys (McCune et al., 1962; Vos and Koeman, 1970); this illustrates the specific action of PCBs in different species. Atrophy of lymphoid organs has been reported in chickens and rabbits exposed to PCBs (Vos 1972). Atrophy of the thymus was pronounced in guinea-pigs (Vos and van Driel-Grootenhuis, 1972), and cellular and humoral immunity was depressed in this species. Monkeys that died from PCBs exhibited liver enlargement, the main cause of death being pneumonia or diarrhoea (Nishizumi, 1970). Hyperplasia and dysplasia of the gastric mucosa was produced in rhesus monkeys by the ingestion of a diet containing 300 mg/kg Aroclor 1248 for three months (Allen and Norback, 1973).

Nishizumi et al. (1969) and Nishizumi (1970) reported an increase of smooth endoplasmic reticulum (SER) in liver cells of mice given a total of 30 mg of Kaneclor 400 over a month. A decrease of rough endoplasmic reticulum was also noticed. Similar changes were found in monkeys.

Nishihara and Yamamoto (1972) reported that proliferation of agranular endoplasmic reticulum was found even two months after a single oral administration of 100 mg/kg of PCBs (Kaneclor 400) to guinea-pigs. In rats, the appearance of the reticulum returned to normal one month after a single administration of 200 mg/kg. Vos and Notenboom-Ram (1972) also noticed the proliferation of the SER in rabbit liver; this resulted in a perinuclear and peripheral displacement of mitochondria and rough endoplasmic reticulum. The focal cytoplasmic hyalin degeneration, as seen under the light microscope, represented densely packed agglomerations of SER in electron microscopic observation.

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7.2.2 Pathological and histopathological changes

7.2.2.1 Induction of hepatic microsomal enzymes

Street et al. (1969) studied the effects of feeding 50 and 10 mg/kg of Aroclor series to female rats for 15 days. The sleeping time with hexobarbital was reduced with 11% Aroclor 1221 (50 mg/kg); with Aroclors 1248 and 1268, the reductions in sleeping time were 35% and 48% respectively. Aniline hydroxylation and demethylation of p-nitroanisole were increased. Induction of microsomal hydroxylating enzymes in the American kestrel (Lincer and Peakall, 1970) and in pigeons (Risebrough et al., 1968) have also been reported.

Fujita et al. (1971) compared the di-, tetra-, penta- and hexachlorobiphenyls, and found that 2,4,5,3',4'-pentachlorobiphenyl was the most potent inducer among the homologues tested.

Villeneuve et al. (1971a) found that the no-effect level of Aroclor 1254 for enzyme induction in the pregnant rabbit was between 1.0 and 10 mg/kg when administered for 28 days during gestation. Aniline hydroxylase and aminopyrine n-demethylase were induced by 10 mg/kg.

Tanaka and Komatsu (1972) studied the effects of small doses of PCBs on microsomal enzymes. The hexobarbital-induced sleeping time in female rats was reduced to 49% of the control value by 2 mg/kg day of Kanechlor 500 for 3 days (total 6 mg/kg). When 0.4 mg/kg day was given for 15 days (total 6 mg/kg), no reduction in sleeping time was observed. When this small dose was continued for 45 and 53 days, the reduction remained at 12-13%. The dose of 0.4 mg/kg day was estimated to be equivalent to feeding 5 mg/kg.

Litterst et al. (1972) fed male rats with 0, 0.5, 5 and 50 mg/kg of Aroclors 1242, 1240, 1254 or 1260 for 4 weeks. The activity of liver microsomal demethylase was increased by feeding 5, 50 or 500 mg/kg of Aroclors 1240, 1254 and 1260, and by 0.5 mg/kg of Aroclors 1254 and 1260. At 500 mg/kg, the increase was by a factor of 2-3. Nitroreductase activity was increased 50-70% by all four Aroclors at 0.5 mg/kg. Aroclor 1260 at 500 mg/kg gave a 10-fold increase. Cytochrome p-450 was increased by all Aroclors at 50 and 500 mg/kg. The activity of glucose-6-phosphatase in the liver decreased following feeding with Aroclors.

Benthe et al. (1972) also studied the induction of oxydative demethylation in rats by a single i.p. injection of 5-500 mg/kg of Aroclor 1232 or 1248. The effect was still evident four weeks after application.

Villeneuve et al. (1972) reported a study on chronic ingestion of PCBs in which the pentobarbital sleeping time of rats was consistently reduced by Aroclor 1254 and 1260.

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7.2.2.2 Hepatic porphyria

Vos and Koeman (1970) reported increased faecal excretion of coproporphyrin and protoporphyrin and fluorescence of tissues in chickens fed technical PCBs (60% chlorinated) at 400 mg/kg. These effects were also found in rabbits exposed to dermal application of 118 mg PCBs for 38 days (total 27 applications) (Vos and Beems, 1971).

Hepatic porphyria was found in rats fed PCBs (Kimbrough et al., 1972) Aroclor 1254 was porphyrogenic when fed to rats at levels of 100 mg/kg (approximately 10 mg/kg day) for four months (Goldstein et al., 1973).

Vos et al. (1971) determined the δ-aminolaevulinic acid (ALA) synthetase activity in relation to hepatic porphyria in Japanese quail given Aroclor 1260 orally for seven days. Liver mitochondrial ALA synthetase activity was increased by 20-fold at the 500 mg/kg level and by 10-fold at the 50 mg/kg level. At the 1 mg/kg level, the increase of activity, as compared with the control, was statistically significant The no-effect level was therefore 0.1 mg/kg (mean liver residue level of PCBs: 10.54 mg/kg) in this experiment.

7.2.2.3 Immunosuppression

Because of the findings of atrophy of lymphoid tissue, lymphopaenia, atrophy of the cortex of the thymus, and reduction in the number of germinal centres in lymph-nodes in experimentally poisoned animals, the immunosuppressive action of PCBs was investigated by Vos and de Roij (1972).

Guinea-pigs were fed Aroclor 1260 at levels of 0, 10 and 50 mg/kg for eight weeks. The serum γ-globulin level was significantly decreased in tetanus-toxoid-stimulated animals fed 10 mg/kg PCBs. As shown by the fluorescent antibody technique, γ-globulin containing-cells in popliteal lymph-nodes were numerically reduced in toxoid-stimulated animals fed 10 and 50 mg/kg PCBs.

In a subsequent study (Vos and van Driel-Grootenhuis, 1972), decreased serum antitoxin titres were recorded in guinea-pigs fed with 50 mg/kg PCBs (Clophen A60 and Aroclor 1260) after immunization with one or two doses of tetanus toxoid. Apart from this effect on humoral immunity, depression of cellular immunity was found at a similar dose level, when delayed hypersensitivity to tuberculin was used as a measure of such immunity. Stress (release of glucocorticoids) was not considered responsible for the reduced immunological response.

Lymphopaenia has been observed in rhesus monkeys fed PCBs (Allen and Norback, 1973) The action of PCBs on the lymphoid system could therefore result in increased susceptability to viral, bacterial or fungal diseases.

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7.2.2.4 Endocrine effects

An oestrogenic activity of PCBs (Aroclors 1221, 1232, 1242 and 1248) was demonstrated by Bitman and Cecil (1970). The oestrogenic activity was evaluated in terms of the 18-hour glycogen response of the immature rat uterus a single subcutaneous injection.

The minimum effective dose was 8 mg. The higher chlorinated mixtures of PCBs were inactive at the 8 mg level. The possibility that the oestrogenic effect of PCBs may be due to in vivo conversion to hydroxylated analogues is supported by the observation that 0,0'-biphenol is a more potent oestrogenic agent than its chlorinated analogues (PCBs).

Komatsu (1972) failed to demonstrate the oestrogenic activity of PCBs (Kaneclor 400, 0.1 g/kg p.o. for 3 days) in castrated rats, but pretreatment of the animals of PCBs potentiated the action of oestradiol in increasing uterine weight in these animals.

Both the increased steroid metabolism, as mentioned by Rehfeld et al. (1971), and the oestrogenic activity could be responsible for the depression of secondary sexual characteristics (decreased development of comb and wattles) noted in cockerels (Platonow and Punnell, 1971). Increased steroid metabolism in pigeon liver homogenates has been demonstrated by Risebrough et al. (1968).

7.2.2.5 Effects on lipid levels

As a result of the findings of elevated lipid levels in the serum of Yusho patients, the effects of PCBs on lipid levels, and particularly on triglyceride levels in plasma and skin lipids, were determined in poisoned animals.

Tanaka et al. (1969) found a ten-fold increase of serum triglyceride level after feeding rats 0.1 g/kg of Kaneclor 400 for four weeks.

Nagai et al. (1971a) gave rats orally 0.5, 2.5, 5 or 50 mg of Kaneclor 400 per animal daily for 2-6 weeks. Plasma triglyceride levels were elevated in the 50 mg group. Plasma cholesterol and phospholipid levels were increased in the 0.5 mg group, and rose still further as the dose was increased. In comparisons between the low-boiling and high-boiling fractions of PCBs, the latter fraction markedly reduced the total lipid content of the abdominal fat tissues and the plasma triglyceride levels.

Rabbits were given p.o. 8 cm^3 of a 1% solution of chlorobiphenyl in olive oil for 3 or 11 days, the lipid composition of liver and plasma was then analysed (Ito et al., 1971). The total lipid and triglyceride levels of liver were raised nearly two-fold after administration of PCBs for three days, while serum triglyceride levels were abnormally increased after administration of PCBs for 11 days.

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Litterst et al. (1972) measured the triglyceride level in liver after feeding rats one of the Aroclor series (from 1242 to 1260) at a concentration of 0, 0.5, 5, 50 or 500 mg/kg for four weeks. The greatest increase in liver triglyceride was found with Aroclor 1248; peak levels decreased as the chlorine content of the Aroclor increased.

Nagai et al. (1972) administered Kaneclor 400 orally to rats at a dose of 1 mg per animal (equivalent to 6.7 mg/kg) for 30 days. At the end of the experiment (1-14C) acetate was injected i.p. to determine the rate of incorporation in lipids. A reduction in free fatty acid and triglyceride in skin lipid was noticed; in addition the incorporation of acetate into triglyceride and free fatty acid was reduced, while that into sterols was promoted. The latter would appear to suggest that chain elongation and disaturation of fatty acids had been activated by PCBs.

To summarize the results of the animal experiments described above, elevation of triglyceride in plasma or serum was found, but only after fairly high doses of PCBs; in contrast, reduction of triglyceride and free fatty acid was found in skin lipid. Further studies are needed to enable these contradictory findings to be explained.

7.2.3 Carcinogenicity

Nagasaki et al. (1972) and Tomii et al. (1973) succeeded in inducing hepatomas in mice by feeding 500 mg/kg of Kaneclor 500 for 32 weeks (7/12 animals, or 58.3%). As shown in table 8, no tumours were found with lower doses nor with Kaneclor 400 and 300.

Ito et al. (1973 personal communication) demonstrated an additive effect in the induction of liver tumours by the simultaneous feeding of 250 mg/kg of Kaneclor 500 and 100 mg/kg of BHC; if the substances were fed separately at these concentrations, no tumours were produced.

Kimura and Baba (1973) induced benign tumours only in female rats by feeding a total amount of 1200-1500 mg Kaneclor 400 for 400 days. The dose of PCBs was increased in stages, starting from 38.5 mg/kg and increasing to 462 mg/kg, and continued at this level for 32 weeks. Pinhead to pear-sized round and pale brown flecks or nodules were scattered on the surface (and also the cut surface) of the liver in the group that ingested more than 1200 mg of Kaneclor 400. Microscopically, multiple adenomatous nodules were found that were interpreted as benign neoplastic lesions.

Male rhesus monkeys were recently fed a diet containing 300 mg/kg of PCBs (Aroclor 1248) or 5000 mg/kg of polychlorotriphenyl (PCT) for 3 months; hyperplasia and dysplasia of the gastric mucosa and invasion of the adjacent tissue region were seen, suggestive of an eventual neoplastic transformation (Allen and Norback, 1973).

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Table 8

INDUCTION OF TUMOURS IN MICE BY PCBs

PCB	Conc. in diet (mg/kg)	No of mice	Liver wt (g)	Nodular hyperplasia	Hepatoma	Amyloidosis
Kaneclor 500	500	12	5 06[a]	+	7/12	0/12
	250	12	3 16	-	0	2/12
	100	12	2 65	-	0	3/12
Kaneclor 400	500	12	3.01	-	0	0/12
	250	12	2.65	-	0	3/12
	100	12	2.80	-	0	10/12
Kaneclor 300	500	12	2 71	-	0	1/12
	250	12	2 11	-	0	4/12
	100	12	2.86	-	0	10/12
Control	-	6	1 45	-	0	0/6

[a] The liver had a rough surface with multiple tumours 0 2-1.0 cm in diameter Some areas of nodules showed an adenomatous pattern Many necrotic foci were seen Nuclear irregularities and mitotic figures were frequently seen in non-tumours areas of the liver (Tomii et al., 1973).

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7 2 4 Effects on subsequent generations

7 2 4 1 Reproductive effects

Reproductive effects are clearly of critical importance for any population. Several investigators have tried to draw conclusions as to effects on reproduction from changes in numbers of individuals over a period of time. Such indirect observations are extremely difficult to interpret, because changes in population size may reflect any of a large number of environmental variables, and the population of animals in a given area may fluctuate naturally to such an extent that it sometimes appears that a species has been eliminated (Longhurst et al., 1972). Studies of effects on reproduction in natural populations must, in most cases, be made directly in terms of parameters such as the number of eggs, hatchability frequency, and survival rate in nests. When performed in a proper way, such observations may significantly contribute to our understanding of the effects of PCBs and other pollutants on populations

In a reproduction study conducted on rats for Monsanto and reported by the Food and Drug Administration (1970), Aroclors 1242, 1254 and 1260 were administered at levels of 1 10 and 100 mg/kg in the diet Aroclor 1242 had no effect on the first generation but, at 100 mg/kg, mating indices were low in the second generation. With Aroclor 1254, the number of pups delivered and the number surviving to weaning were reduced in both the second and third litters. Aroclor 1260 was found to affect reproduction at 100 mg/kg, increasing the number of stillborn animals. No effect was observed at levels of 1 and 10 mg/kg

More recently, low mating indices and decreased survival of pups were reported for animals receiving Aroclor 1242 at 100 mg/kg, and decreased survival of pups for rats receiving Aroclor 1254 at 100 mg/kg. No reproductive effects were observed with Aroclor 1260 at 1, 10 or 100 mg/kg, or with Aroclor 1242 or 1254 at 1 or 10 mg/kg (Keplinger et al., 1971).

Placental transfer of PCBs into the developing foetus was demonstrated when Aroclor 1221 and 1254 were administered orally to pregnant rabbits The foetal liver showed higher residue levels of PCBs than the maternal liver (Grant et al , 1971a).

Aroclor 1254 administered orally to rabbits at a dose of 12.5 mg/kg day during the first 28 days of pregnancy induced abortion and was foetotoxic. Dead foetuses from treated rabbits showed no consistent skeletal abnormalities (Villeneuve et al., 1971b).

Aroclor 1254 was given orally to pregnant rats once daily from day 7 to day 15 of organogenesis (Curley et al., 1973). Dose levels were 0 10 and 50 mg/kg day. No statistical difference was found between control and dosed groups with respect to the total weight of litters, the percentage of pups born dead, or the survival rate to weaning. Liver enlargement was observed in weanlings from dosed rats There was a significant

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increase in the relative liver weights of weanlings from dosed rats compared with those from controls, and the livers of most of the exposed weanlings contained enlarged hepatocytes, accompanied in some cases by cytoplasmic vacuolization and bile-duct proliferation, particularly in the group given the higher dosage

In feeding trials with PCBs in mink, at a concentration of 30 mg/kg (10 mg/kg each of Aroclor 1242, 1254 and 1260), Ringer et al. (1972) observed a strong depression in the breeding potential. Mink appeared to be quite sensitive to PCBs

Levels of PCBs and of DDE in sea lions (Zalophus californianus) in Southern California that have a high rate of premature births are higher than in those that produce full-term healthy pups (de Lang et al , 1973). The effects of PCBs cannot be separated from those of DDE in this instance, but both PCBs and pp'-DDT have been shown to prolong the length of the oestrous cycle in mice (Orberg et al., 1972; Kihlström et al., 1973) at the concentrations found in the sea lions. Effects on the levels of circulating oestrogen are thesefore suggested, probably caused by an elevated rate of metabolism of oestrogen and of other steroid hormones by hepatic detoxifying enzymes induced by PCBs, DDE and a number of other environmental contaminants (Benthe et al., 1972; Litterst et al., 1972). A disruption of normal hormone levels is a plausible cause of the premature births observed among the sea lions of California.

In both Europe and North America, a number of species of raptorial fish-eating birds have suffered local reproduction failures In part, these have been associated with thinning of the egg-shells caused by pp'-DDE. PCBs might contribute to the reproductive failures, however, together with the chlorinated hydrocarbon insecticides, through the induction of hepatic enzymes. The resulting higher rates of hormone metabolism could cause an imbalance that would interfere with reproduction.

Epidemiological evidence suggests that PCBs are affecting the reproduction of the Atlantic salmon (Salmo salar) in Sweden. Concentrations of PCBs of 8 and 15 mg/kg in the lipid of ova are associated with egg mortality rates of approximately 30 and 40% respectively The mortality approaches 100% as the levels reach 20 mg/kg (Johansson et al , 1970). Mean levels of PCBs in salmon in the Baltic are currently about 13 mg/kg in the lipid; in addition, DDT compounds are also present in concentrations approximately twice those of the PCBs (Jensen et al., 1972).

Fish from the Great Lakes of North America have traditionally been fed to domestic mink in nearby states In the mid-sixties, reproduction began to decrease. In particular, when breeding mink were fed exclusively with the coho salmon, reproduction ceased completely, either as a result of embryonic mortality or of the death of newborn kits (Aulerich et al., 1971) Experimental work has shown that the mortality was caused by contamination of the fish with PCBs. Adult mink were also found to be sensitive to PCBs; mortality increased on feeding either Lake Michigan coho salmon or ocean fish supplemented with 10 mg/kg each of

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Aroclors 1241, 1248 and 1254 (Ringer et al , 1972) Levels of PCBs in the captive mink that died of poisoning were 11 mg/kg, 4-5 mg/kg and 5 mg/kg in the brain, liver and muscle respectively. Concentrations of this magnitude are currently present in other mammals that feed on fish (e.g , wild mink and seals). Nothing is known, however, about the sensitivity of these species to PCBs, and experiments under controlled conditions are difficult to carry out

7.2 4 2 Teratogenicity

Teratogenicity studies have been conducted in rats with Aroclor 1242, 1254 and 1260 (Keplinger et al., 1971). Daily oral doses of 10 and 30 mg/kg on days 6 to 15 of gestation produced no observable effects.

Teratogenic effects were noted when 5 mg of Aroclor 1242 was injected into the yolk sac of chicken eggs (McLaughlin et al., 1963).

7.2 4.3 Mutagenicity

Embryos from the second generation of ring doves fed 10 mg/kg of Aroclor 1254 exhibited a high frequency of chromosomal aberrations (Peakall et al., 1972). This level of dietary PCBs was also associated with a high incidence of embryonic deaths. A dominant lethal assay in rats gave no evidence of mutagenic effects (Keplinger et al., 1971).

No chromosomal aberrations were observed in human lymphocyte cultures exposed to Aroclor 1254 at 100 mg/kg (Hoopingarner et al., 1972)

7 3 Minimum effect levels

In long-term chronic feeding tests on rats carried out at the Industrial Biotest Laboratory, the minimum effect level (on livers of mothers and on pups) was 100 mg/kg of Aroclor 1260 in the diet (probably equivalent to 5 mg/kg day).

Villeneuve et al. (1971a) found a minimum effect level for enzyme induction in the pregnant rabbit at 10 mg/kg Aroclor 1254, when administered daily for 28 days during gestation.

Lincer and Peakall (1970) fed kestrels for five months with Aroclor 1254 and 1262 at levels of 0.5 and 5 0 mg/kg, the lower dose being roughly equivalent to 0 2 mg/kg of PCBs. A dose-dependent in vitro breakdown of oestradiol to a more polar metabolite occurred in the livers of kestrels fed either Aroclor 1254 or 1262. No such conversion took place in the livers of the control birds. The increase in hepatic enzyme activity correlated with an increase in cytoplasmic RNA.

Vos et al. (1971) measured the activity of the mitochondrial enzyme ALA-synthetase in the livers of Japanese quail. Significantly increased activity was found at a minimum effect level of 1 mg Aroclor 1260/kg,

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administered daily for seven days. The content of PCBs in the liver at that dose was 1 41 mg/kg.

Calculation of tolerances for PCBs in foods or of acceptable daily intakes of PCBs in human diets is theoretically possible on the basis of the minimum effect levels given above. For the time being, a figure in the range 1-3 µg/kg day (70-210 µg/day for an adult) is suggested as reasonable for an Acceptable Daily Intake (Environ. Res , 1972) An ADI of 210 µg/day if assumed to be accumulated over 240 days, would give a safety factor of approximately 10 as compared with the lowest cumulative doses estimated as producing an overt effect in the Yusho incident (Kuratsune et al., 1972). However, toxicological evaluation of the significance of residues of PCBs in food by extrapolation from results obtained in animal experiments is dangerous and perhaps not justified, since the composition of the different mixtures of PCBs present in food is different from that of commercial mixtures In addition, the various isomers may differ in toxicity For this reason, there is insufficient information for the establishment of an Acceptable Daily Intake for man. To solve this very complicated problem, toxicity studies of the different compounds present in mixtures of PCBs are necessary, as well as the identification and quantitation of the individual compounds present in food. The matter is further complicated by the lack of knowledge as to the fate of toxic impurities in the environment

8 EPIDEMIOLOGICAL AND CLINICAL STUDIES

8 1 Rice bran oil poisoning (Yusho)

In early October 1968, sporadic outbreaks of a peculiar skin disease were noticed in northern Kyushu district (west Japan). A special Study Group was organized in Kyushu University in order to study this disease. The group named the disease "Yusho" (oil disease), and the experimental, clinical and epidemiological findings on the disease were collected and published by the group in three monographs (Study Group for "Yusho", 1969; Study Group for the therapy of "Yusho", 1971, 1972).

The cause of the disease was found to be the consumption of a brand of rice bran oil contaminated by Kaneclor 400 (the principal component being tetrachlorobiphenyls) in the heat exchanger used for refining the crude oil

The clinical picture included acneiform eruption, distinctive hair follicle, pigmentation of skin, nail, conjunctiva and oral mucosae, and hypersecretion from meibomian glands The systemic symptoms included anorexia, general fatigue, weight loss and impotence. Based on the

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dermatological findings, a clinical grading was proposed by Goto and Higuchi (1969) (table 9).

Table 9

CLINICAL GRADING OF POISONING BY PCBs

Grade	Definitive findings	Other findings
I Faint	Cheese-like discharge from meibomian glands, dark brownish pigmented nails	Hyperhydrosis of the palms, gingival pigmentation
II Slight	Comedoform skin eruption	Follicular keratosis in joint areas and extensor surface of extremities
III Moderate	Acneiform skin eruption, alterations in hair follicles of neck or chest	Oedema of eyelids, bursa-like cyst formation in joint areas
IV Severe	Widely distributed acneiform eruption, distinct follicles on almost entire surface of skin	Swelling of face or lower extremities, severe secondary infection of skin eruptions

8.2 Biochemical findings

The serum lipid level, and particularly the triglyceride fraction, was elevated (Uzawa et al., 1969). Serum triglyceride ranged from 200 to 600 mg% in 50% of the patients, while the serum total cholesterol remained unchanged and phospholipid tended to be somewhat lowered. This elevated level of serum triglyceride did not decrease significantly during the 3 years following the poisoning (Uzawa et al., 1971, 1972). The levels of serum alkaline phosphatase and transaminases were found to be slightly increased in the patients, indicating a minor disturbance in liver function. The urinary total 17-ketosteroids and 17-hydroxycorticosteroids were determined in 50 male and 45 female patients, and in 42% of the patients, the normal range of both steroids was exceeded (Nagai et al., 1971b). The fatty material contained in Yusho acne was shown to differ from that of ordinary acnes in fatty acid composition The former contained more stearic and oleic acids than did non-Yusho acne Linoleic acid was present only in the former, which also contained 10 times more cholesterol than the latter (Nagai et al., 1969). The porphyrin level in the blood and urine of Yusho cases has not been examined

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8 3 Biopsy findings

A decrease of rough endoplasmic reticulum and hypertrophy of smooth endoplasmic reticulum were observed in the liver of a Yusho patient Mitochondria showed morphological heterogeneity and giant mitochondria were frequently seen. Many filamentous inclusions were present in the matrix (Hirayama et al., 1969; Yamamato et al., 1971).

Biopsy speciments from 18 Yusho patients showed a marked hyperkeratosis and cystic dilation of hair follicles, and a marked increase of melanine in the basal cells of the epidermis (Kikuchi and Hashimoto, 1969) Abnormal pigmentation of the conjunctiva was one of the characteristic signs of Yusho patients An electron microscopic examination of the pigmented bulbar and forni conjunctivas showed that a large number of melanin granules were present in the cytoplasm of the epithelial cells, especially in that of the basal cells of the conjunctiva. Apart from the melanin granules, innumerable tiny electron-dense particles of diameter of 30-40 mm were distributed diffusely in the cytoplasm of the basal cells (Ikui et al., 1969).

8.4 Neurological findings

In 9 of 23 patients examined, sensory nerve conduction was found to be decreased in the radial and sural nerves, while motor nerve conduction was shown to be unaffected in the ulnar and tibial nerves, except in two patients in whom it had decreased (Kuroiwa et al., 1969). Electroencephalographic examinations were made in 9 of 20 Yusho patients A slightly abnormal tracing with a low-voltage 6-7 c/s theta wave of frontal dominant was observed in one patient but was not considered to be caused by Yusho (Nagamatsu and Kuroiwa, 1971).

8 5 Immunological findings

Immunoglobulin levels in 149 samples of serum from 72 patients (59 adults and 13 children) were determined 2 to 4 years after the poisoning. IgA and IgM levels in serum were found to be decreased, and IgG levels increased, in 1970. They had returned to the normal range of values in 1972, except in a few cases in which IgA still remained lower than normal. IgA levels in a few cases with respiratory symptoms were less than 50 mg/dl, suggesting decreased resistance to respiratory infection, although no statistically significant relationship between IgA level and clinical symptoms was found. IgM levels were significantly lower in the patients with severe dermatological symptoms IgA levels in sputum were also examined in 9 patients, and were not found to be lowered (Saito et al., 1972).

8 6 Yusho babies

Thirteen women (11 with Yusho and 2 unaffected wives of patients) delivered 10 live-born and 2 still-born babies in 1968. Nine of them had unusually grayish, dark-brown skin, and similar pigmentation was noted

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in five of them (Taki et al., 1969; Yamaguchi et al., 1971; Funatsu et al., 1971).

Histological examination of a still-born foetus showed marked hyperkeratosis and atrophy of the epidermis, a marked increase of melanin pigments in the basal cells of the epidermis, and cystic dilation of hair follicle. In several cases, a tiny milk tooth had erupted. Twelve of 13 foetuses were smaller than the national standards and 4 of them were small-for-dates babies. As they grew older, the discoloration of the skin gradually faded. No evidence is available as to any physical or mental retardation of the babies

8 7 Treatment

No satisfactory treatment for Yusho has yet been developed in spite of the efforts of the Yusho Study Group and others during the past five years. Imamura (1972) tried a fasting therapy on 20 Yusho patients with persistent neurological and dermatological symptoms. The therapy consisted of complete fasting, water alone being given for two days, and fruit and vegetable juices for the subsequent 6-12 days. Milk was also supplied occasionally. He observed a marked improvement in some patients with stubborn headache, bronchitis or emesis. This therapy, in general, was effective for the neurological symptoms of Yusho, whereas the dermatological symptoms were more resistant. It was also reported by Toshitani and Kitamura (1971) that the local application of a vitamin A acid lotion was effective in removing keratotic plugs of acne, leading to a significant improvement in the external appearance of the patients.

8,8 Follow-up observations

Over a period of five years, the initial severe symptoms have gradually disappeared in most cases, but some patients are still suffering from chloracne and enlarged follicular glands excreting a cheese-like material. Toshitani (1972) compared the status of skin lesions of patients in 1971 with that in 1969, and found that 33% of the subjects showed some improvement while 20% had become worse; the remaining 47% showed no appreciable changes at all. He noted that acneiform eruptions tended to persist in covered areas, such as the pudendal area, while some improvement was seen in exposed areas, such as the face. Various systemic subjective symptoms, such as fatigue, general malaise, stubborn headache, nausea, or numbness in the limbs, were frequent and seriously disturbed the rehabilitation of patients. Shigematsu et al (1971) reported the striking fact that about 40% of the patients were troubled with persistant cough with expectoration, and showed X-ray and bronchographic findings similar to those of chronrc bronchitis even 2 to 3 years after the poisoning. Such symptoms were often found particularly in children. The growth of children has been shown to be retarded. Growth of teeth or absence of permanent teeth was occasionally observed in Yusho children, suggesting that some disturbance in calcium metabolism was caused by intake of PCBs (Ueda et al., 1973, personal communication).

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9. MAXIMUM PERMISSIBLE LEVELS AND POSSIBLE REMEDIAL ACTION

9.1 Maximum permissible levels

On the basis of long-term inhalation tests on experimental animals, the maximum allowable concentration in the air of a work-place (threshold limit value; TLV) has been set in the United States of America as 1 mg/m^3 for PCBs containing 42% chlorine, and 0.5 mg/m^3 for PCBs containing 54% chlorine. These values of the TLV were selected mainly to ensure the prevention of chloracne.

9.2 Replacement by new products

It is difficult to predict what compounds might be used as substitutes for PCBs. Of the chlorinated hydrocarbons, polychlorinated terphenyls (PCTs), chlorinated naphthalenes (CNs) and long-chain chlorinated paraffins (CPs) are the known possible candidates. The determination of PCTs and CNs will present problems similar to those encountered with PCBs. PCTs have already been detected in environmental samples (Zitko et al., 1972; Freudenthal and Greve, 1973; Mestres and Illes, 1973).

CNs have not been reported in environmental samples thus far; it is likely that small amounts of CNs would not be detectable by the present methods in the presence of a large amount of PCBs.

The determination of CPs in biological samples is quite different from that of PCBs. Gas chromatography cannot be used and it is necessary to base the quantitation on the determination of chlorine, for example, by pyrolysis and microcoulometry. A clean-up procedure and a confirmation technique for CPs has recently been described (Zitko, 1973, 1974), but CPs have not yet been detected in environmental samples.

Polybrominated biphenyls might be used as fire-retardants (Michigan Chemical Corporation, 1971), but it is not likely that they will be used on a very large scale.

Some organophosphates are being used as substitutes for PCBs. The analytical techniques for these compounds will be similar to those used for organophosphorus pesticides.

Alkylated biphenyls and organosilicones could also be used instead of PCBs. Analytical methods for aromatic hydrocarbons are well established and could be used for the determination of alkylated biphenyls. There is no information on the determination of organosilicones in environmental samples.

9.3 Conclusions

1. Interpretation of the results of toxicity studies is complicated by the possible contamination of mixtures of PCBs with toxic impurities.

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2 The toxicity of PCBs could probably be substantially reduced by the preparation of PCBs that are free from toxic impurities, such as chlorinated dibenzofurans

3 There is no simple relationship between the toxicity of PCBs and: (a) the degree of chlorination; or (b) substitution patterns It is therefore not possible to conclude now that one type of preparation is less toxic than another

4 Large species differences exist in the pathological effects produced by particular PCBs (e.g . degree of liver damage, hepatic porphyria, oedema formation, acnegenic skin lesions).

5 PCBs or their metabolites may produce effects at low doses (microsomal enzyme induction, porphyrogenic action, oestrogenic activity, immunosuppression).

6. In man, accidental poisoning with PCBs produced Yusho disease, associated with a peculiar effect on skin (chloracne, pigmentation of the skin and nails, and hypersecretion by meibomian glands), slightly altered liver function, elevated blood-serum triglyceride, and other biochemical and pathological lesions.

7. The estimation of an Acceptable Daily Intake for man is not possible at the present time, since the composition of the different mixtures of PCBs present in food differs from that of commercial mixtures used in animal experiments

8 Although there is no evidence of overt disease or detectable health impairment from the body burdens of PCBs found in the general population at the present time, the possibility of more subtle effects cannot be eliminated. However, it is clear that, in an infant fed on contaminated human milk, as found in certain areas, a level almost one-fifth that associated with clinical symptoms will be reached Accordingly, further increase in sources of human exposure is to be strongly discouraged.

9 4 Recommendations

1 The methods of identification of individual PCBs and associated impurities in commercial mixtures and of their metabolites and degradation products should be improved, and comparability of analytical results ensured.

2 Pure compounds and contaminants likely to be sources of exposure for man should be prepared in order to refine analytical techniques, carry out toxicological studies, and more precisely evaluate toxicological data and the problem of interactions

3 Experimental studies of toxicity, mode of action, kinetics of uptake and elimination, and chronic studies should be carried out with selected pure isomers; these should cover porphyrogenesis, carcinogenesis,

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teratogenesis, mutagenesis and immunosuppression. Special attention should be paid to toxicity studies on the foetus and newborn Biological interations of components of mixtures of PCBs with chemical or microbiological agents should be explored

4. Non-toxic substitutes for PCBs should be sought and fully studied for their toxicological effects on human health and the environment This approach must be adopted with all new chemicals developed for widespread use Related compounds, such as the PCTs and brominated biphenyls, should not be used until their health and environmental effects have been carefully evaluated,

5. Population sub-groups that have been accidentally or occupationally exposed should be studied epidemiologically to establish the presence or absence of health impairment, as well as the kinetics of uptake, storage and elimination of PCBs; the studies should cover porphyria, and immunosuppressive, carcinogenic, teratogenic and mutagenic effects. Population exposure should be assessed by the measurement in man of the levels in body tissues, human milk, blood, adipose tissues and lipid fractions. In these determinations, the fat content of blood must be determined Determinations of components of mixtures of PCBs in the food chain are also required

6. In all uses of PCBs, the presence of toxic impurities should be controlled

7 The reporting and publication of any new cases of accidental poisoning with PCBs in the human population is to be encouraged.

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