STATENS NATURVÅRDSVERK
The National Swedish Environment Protection Board
Research Secretariat.

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P R O G R A M

for

P C B CONFERENCE II

December 14, 1972

in the "Wenner Gren Center",
Session Hall, Annex Building,
166 Sveavägen,
STOCKHOLM, S w e d e n

Chairman: LARS FRIBERG, Prof., M.D.,
National Swedish Environment Protection Board, Solna.

0900-0910 "Introduction"
LENNART HANNERZ, Ph.D., Director of Research,
National Swedish Environment Protection Board, Solna.

0910-0940 "How reliable are the different PCB quantitation methods?"
SÖREN JENSEN, Ph.D.,
National Swedish Environment Protection Board, Solna.

0940-0955 "PCB in air borne fall-out",
ANDERS SÖDERGREN, Ph.D.,
University of Lund

0955-1010 "PCB:s possible health hazards", (after lunch)
MATHS BERLIN, Prof., M.D.,
University of Lund.

1010-1020 Discussion

C o f f e e b r e a k

(continued over leaf)

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1045-1105 "PCB in the Finnish environment",
MATTI HELMINEN, Ph.D.,
Finnish Game and Fisheries Research Institute, Helsinki, (Finland).

1105-1135 "Monitoring PCBs in Water and Wildlife",
ALAN HOLDEN, B.A.,
Freshwater Fisheries Laboratory, Pitlochry, (U.K.).

1135-1200 "PCB in mammals and birds in the Netherlands",
JAN H. KOEMAN, Prof., Ph.D.,
Landbouwhogeschool, Wageningen, (Holland).

1200-1215 "PCB in some Swedish mammals and birds",
TJELVAR ODSJÖ, B.Sc.,
Museum of Natural History, Stockholm.

1215-1230 "PCB in aquatic nourishment webs",
MATS OLSSON, B.Sc.,
Museum of Natural History, Stockholm.

1230-1245 Discussion

Lunch 12 30 - 1, 45.

1400-1415 "Synthesis of ^{14}C-labelled and unlabelled PCB-compounds",
CARL-AXEL WACHTMEISTER, Ph.D.,
University of Stockholm.

1415-1430 "Distribution and metabolism of PCB in mice and birds",
BENGT MELVÅS, Ph.D.,
University of Gothenburg.

1430-1440 "Metabolism and distribution of PCB (pentachloro-) in the mouse",
JOHN GAGE, D.Sc.,
University of Lund.

1440-1455 "PCB - effects on mammalian reproduction",
JAN ERIK KIHLSTRÖM, Ph.D.,
University of Uppsala.

1455- General discussion

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PCB Conference II

Stockholm den 14 december 1972

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PCB Conference II
Stockholm 1972

PROBLEMS IN THE QUANTITATION OF PCB IN BIOLOGICAL MATERIAL

Sören Jensen, Lars Renberg and Reggie Vaz
National Swedish Environment Protection Board
Special Analytical Laboratory

Seldom have analysts faced greater problems than those encountered in the quantitation of PCB!

We have therefore found it important to draw especially our biologists attention to the problems attached to the results obtained from a PCB analysis. It is maybe necessary to pause for a moment and discuss the complexity of the number of substances involved which are to be identified and quantitated with the help of a gas chromatograph.

As well known PCB consists of a large number of homologues and isomers of chlorinated biphenyls. The number of chlorine atoms per molecule usually varies from 4 to 8. A technical product like Clophen A 50 contains 50% chlorine and the corresponding gas chromatogram, shown in Fig. 1a, results in 14 peaks. However, we do know that the number of isomers is much larger. It has now been shown, through the use of capillary columns, that the number in common PCB formulations is at least 69.[1] The normally used gas chromatographic columns are not only good enough because they can not separate the PCB s, but also because DDT, and its metabolites DDE and DDD, normally present in biological material, are not separated completely from the PCB s. If the presence of the o,p isomers is also considered, there are another 6 substances, whose peaks appear within the same retention times as those of the ten main components of PCB.

Fig. 1 shows (a) a chromatogram of PCB (Clophen A 50), (b) DDT and its metabolites and (c) a mixture of PCB and DDT with its metabolites, the last illustrating a most complicated chromatogram.

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Fig. 2 shows two chromatograms of biological samples. PCB components in the herring sample are difficult to quantitate due to the high content of DDT and its metabolites, while the contrary is true for the plankton sample.

The problems we meet during the analysis of PCB in biological material fall within two groups. Firstly, the samples contain besides PCB other chlorinated substances of different origin. Secondly, the complexity of the PCB chromatogram can be responsible for uncertain results even in those cases where a pure PCB extract is obtained.

An analysis of PCB consists of two stages 1) working up of the sample for injection into the gas chromatograph. 2) Quantitation of the PCB chromatogram.

The work before a sample is ready for injection into a gas chromatograph can be divided into four steps: extraction of the homogenized sample, determination of the fat contents, clean-up, and separation. The last step - separation - can in certain cases be omitted, since the gas chromatograph besides quantitation also carries out a separation.

Extraction of the lipophilic substances are performed by different methods which may vary from laboratory to laboratory. Two risks are involved:
a) the extraction is not sufficiently effective. A conventional recovery experiment is not satisfactory as it is built on a post mortem addition of a standard. The extraction is then assumed to be as effective as that of pure biological samples. This is of course not true. It is easier to extract the former than the latter. Only if radioactive marked test substances have been added to the animal alive, a relevant recovery experiment can be carried out.

The next problem is: b) the fat weight determination. When the amounts of PCB have been estimated, they have to be related to the weight of the sample. Normally this is done on a fresh tissue basis, but from an ecological point of view, a calculation based on the fat weight is valuable.

The risk is that different fat determination methods give different results. This implies that results can vary from laboratory to laboratory, since a factor, in the fat weight determination, will result in an error of the same factor in the PCB estimation. It is therefore essential to relate the fat weight determination to some official method like the Torrey method,

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where the extraction is performed using a mixture of chloroform and methanol. The solvents normally used for extraction have earlier mainly been ether-hexane mixtures. They have tended to give lower fat weight values than the above mentioned Torrey method.

Nowadays a dehydrating solvent, for example, acetone or acetonitrile is also added, which serves to open the cells for extraction.

Theoretically, the fat determination can be carried out as an independent analysis. However, it is common to use a gravimetric method, that is to say, the solvent is evaporated from the extract and the rest, which is defined as fat, is then weighted.

When small amounts of fat are to be determined, the errors in a gravimetric method become so large, that a more advanced method has to be used (e.g. a spectrophotometric method).

The clean-up methods can be divided into two groups, partly methods, which result in a separation at the same time - chromatographic methods - and partly those where the fat is removed by a single chemical treatment.

The concepts "clean-up" and "separation" sometimes become synonymous because the same step is often responsible for both clean-up and separation. When for example a fat extract is to be purified on a silica gel column, this constitutes a clean-up, that is, the removal of substances that are not to be studied, but at the same time a separation of substances occurs (like PCB from DDT), each of which are to be evaluated.

Two types of chemical treatment for the removal of fat are employed at our laboratory, the one of alkaline hydrolysis, the other by treating the sample with sulphuric acid[2]. In the alkali treatment a conversion of DDT to DDE and DDD to DDMU takes place, besides hydrolysis of the fat. This type of clean-up is of course not suitable when the sample is to be quantitated both with respect to DDT and its metabolites as well as PCB.

Shaking the extract with concentrated sulphuric acid (containing 7% sulphur trioxide) has proved to be a quick and simple method. A disadvantage is that certain pesticides (dieldrin among others) are destroyed by this treatment, consequently this method can not be used for the analysis of PCB

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and such pesticides. Neither DDT nor its metabolites are affected by the sulphuric acid treatment, and since these pesticides are of major importance in samples from Sweden, we normally use this method because of its quickness and simplicity.

The non destructive methods used for clean-up (and separation) consist of chromatographic methods, usually column chromatography or thin layer chromatography. The different principles which have come to use are adsorption chromatography, partition chromatography and gel permeation chromatography.

A comparison between the technical procedures in adsorption and partition chromatography suggests that thin layer chromatography is in most cases more suitable than column chromatography. Better separation, quicker techniques, simpler procedures are some of the advantages of thin layer chromatography.

The solid phase in adsorption chromatography usually consists of silica gel, aluminium oxide, celite, Florisil, etc. or mixtures of these. It is important here to achieve good reproducibility. In adsorption chromatography the substances are separated in order of their polarity. Using a column, this means that non-polar substances are eluted first (like PCB and DDE). This is normally done using hexane, heptane, etc. Changing to a slightly more polar solvent results in the elution of the more polar pesticides (like DDT) while the fat remains on the column. Using thin layer chromatography this implies a high R_f - value for PCB and DDE, while low values for the fat components.

One disadvantage with adsorption chromatography is that the reproducibility and separation is affected by varying fat contents. To obtain the best possible separation it may therefore be necessary to remove the fat components by another method e.g. partition between a non-polar and a polar solvent (usually hexane-acetonitrile).

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Partition chromatography is less usual. Paraffin or a silicon polymer is normally used as the stationary phase. Another method involves acetonitrile-impregnated silica gel and hexane as the elution solvent. The fat is here first eluted, and then the chlorinated hydrocarbons.

Gel permeation is a new method for clean-up based on the separation of molecules according to their size.[3] Here the larger fat molecules are eluted first and then the lower molecular compounds (PCB, DDT, etc.). An advantage with this method is that the column can be re-used, with high reproducibility. High fat contents create no problems.

A specific problem is the separation of PCB from DDT and its metabolites. If we look at Fig. 1c, 2a and 2b, we observe that DDT, DDD and DDE interfere with certain PCB peaks. PCB can be separated from DDT and DDD, through adsorption chromatography.

To separate DDE from PCB by purely chromatographic methods seems on the other hand very complicated. Different methods have been suggested in litterature, but we have not found them suitable for use in routine analysis. It has been reported that PCB can be separated from DDE by highly activated silica gel.[4] We have not succeeded, after repeated trials at our laboratory, to separate DDE from all PCB substances according to this method. In the DDE fraction some PCBs are still found. Unfortunately, in the gas-chromatographic analysis DDE will interfere with these PCBs.

However, methods exist whereby PCB can be obtained free from DDE. Treatment with potassium dichromate, dissolved in sulphuric acid, has been reported to remove DDE, without altering the PCBs.[5] Another oxidation method involves the use of chromic trioxide in acetic acid. This will convert DDE to dichloro-benzophenone, which can be separated from PCB by adsorption chromatography.[6]

After the extract is properly cleaned-up and interfering compounds have been removed, the next problem arises:

How is the extract, containing only PCB, to be quantitated, using a gas-chromatograph with an electron capture detector?

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The widely different methods,used for the quantitation of PCB, illustrate well the complex nature of the problem of calculating the PCB content in biological samples. There are several reasons as to why there does not exist an universally accepted method. The main problem is that no standard formulation, can be directly used to estimate PCB in biological material. It has been shown that the PCBs, present in different types of biological samples, do not correspond in every detail to commercial formulations.

Another difficulty is that most gas chromatographic columns used do not yield complete resolution of the PCB components. Different columns also result in different chromatograms.

In view of these complications, analysts use different quantitation methods. A few of the practiced methods are:

1) Quantitation of the individual components from the PCB chromatogram

In this method a standard formulation is used where each component of the standard, separated on a gas chromatograph, is quantitated individually.

A commercial formulation that most resembles the PCB pattern in biological material is choosen as the standard. This formulation is quantitated 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 is determined. With the same gas chromatographic system attached to a microcoulometric detector the amount of chlorine in each peak is estimated. By combining the qualitative and quantitative information thus obtained, the concentration of each component in the standard is determined. Fig. 1a shows a Clophen A 50 solution injected into a column, comprising of 67 parts 8 % QF 1 and 33 parts 4 % SF 96, mechanically mixed. This chromatogram contains 14 peaks.

The peak height of each component in the standard is compared to the respective peak height in the sample and the amount of each component calculated. The amounts are then added to give the total PCB content in the sample.

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The above method is used for routine analysis at our laboratory.[2] It is advantageous since individual components are estimated. It is surprising that hardly any publications give data on the individual components determined. Only total PCB contents are named. This seems accepted while on the other hand DDT and its metabolites are nearly always reported individually.

A disadvantage with this method is that quantitations are restricted to the same column as that on which the standard formulation has been determined. The use of other columns result in different chromatograms, comprising of more or less than 14 peaks.

Good gas chromatographic separation is demanded. This implies that support material and stationary phase must be handled well to give a high number of theoretical plates. The procedure needs the measurement of many peaks. This difficulty can be solved by reliable automatic integration, coupled to a data system, to speed up quantitation.

2) Quantitation on the assumption that the PCB amounts can be estimated by using the response of DDE as a reference. Here the total peak area of the sample is directly compared to that of a p,p'-DDE standard. Another version consists of multiplying each individual peak height by the corresponding retention time and summing all the products so obtained. This sum is then divided by the product of the peak height and retention time for p,p'-DDE to give the amount of PCB.[7]

The electron capture response for chlorinated biphenyls with 4-9 atoms of chlorine per molecule (normally found in biological samples) is reported not to exceed the value 1.6 relative to p,p'-DDE.[8] The assumption of the similarity in response of each PCB component to that of p,p'-DDE is questionable. Although this method is said to give reliable values for the total PCB content, it must be erroneous when individual component amounts are desired.

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3) Quantitation based on an average of certain peaks when similarity to a certain PCB formulation is assumed. In this quantitation the chromatogram of the sample is studied, and the best resemblence to a standard PCB formulation is examined. Once this standard is decided, a peak from the sample is compared to the corresponding peak in the standard, and thus the whole PCB content is estimated. If several peaks are considered an average is then calculated. This method is rapid and ideal when the PCB chromatogram of the sample is identical to that of the standard. It is, however, well known that the chromatograms of the biological samples seldom resemble those of the standard formulations used. This maybe explained by assuming that the samples contain a mixture of PCBs from different sources. The fact that degradation of certain components can occur and is suspected to be true for some of the chlorinated biphenyls gives this method less reliability. Selective accumulation of different chlorinated biphenyls constitutes also a source of error. The choice of representative peaks can be problematic.

4) Quantitation based on the single peak obtained after total chlorination of PCB. Recently, it has been reported[9] that samples analyzed for PCB have, after clean-up, been treated with antimony pentachloride. The PCB components are thus chlorinated to decachloro-biphenyl. The chromatogram, consisting of a single peak, is then compared to a standard similarly treated.

This method calls for effective clean-up and separation prior to gas chromatography. It demands a reproducible chlorination procedure. The reported yields are stated to be around 85%[9]. With this method, quantitation of the individual PCB components is not possible.

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We have here dealt with different methods for the separation and quantitation of PCB. These can, of course, be combined to attain the highest possible certainty in the PCB determination. However, it is questionable whether the highest degree of certainty always is desirable. An analytical method involving many stages will restrict the number of analysis a laboratory can carry out. It may therefore be necessary to choose between analyzing a few samples with the highest degree of certainty, or to have a large number of analysis performed with a slightly less certainty. This will naturally depend on the aim of the analysis.

We know that biological accumulation can vary a great deal for animals under identical conditions. This variation must be even larger in nature where animals live under different conditions. It is therefore important, from a statistical point of view, that a sufficiently large number of analysis are performed. This may imply that the highest degree of certainty can not be maintained for each sample.

Lastly, we would like to stress the importance of publishing detailed information of the methods used for PCB determination. Astonishingly, very few reports describe fully the quantitation methods used. A direct comparison of PCB values in different reports is thus rendered difficult.

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Regarding the Figures

The gas chromatographic conditions were
Injector temp: 210°C
Column: 160 cm x 1,8 mm, filled with 2 parts 8 %
QF 1 + 1 part 4 % SF 96 on silanized Chromosorb W
100/120 mesh.

Columntemp: 185°C
The detector (electron capture Ni 63) was held at 220°C.

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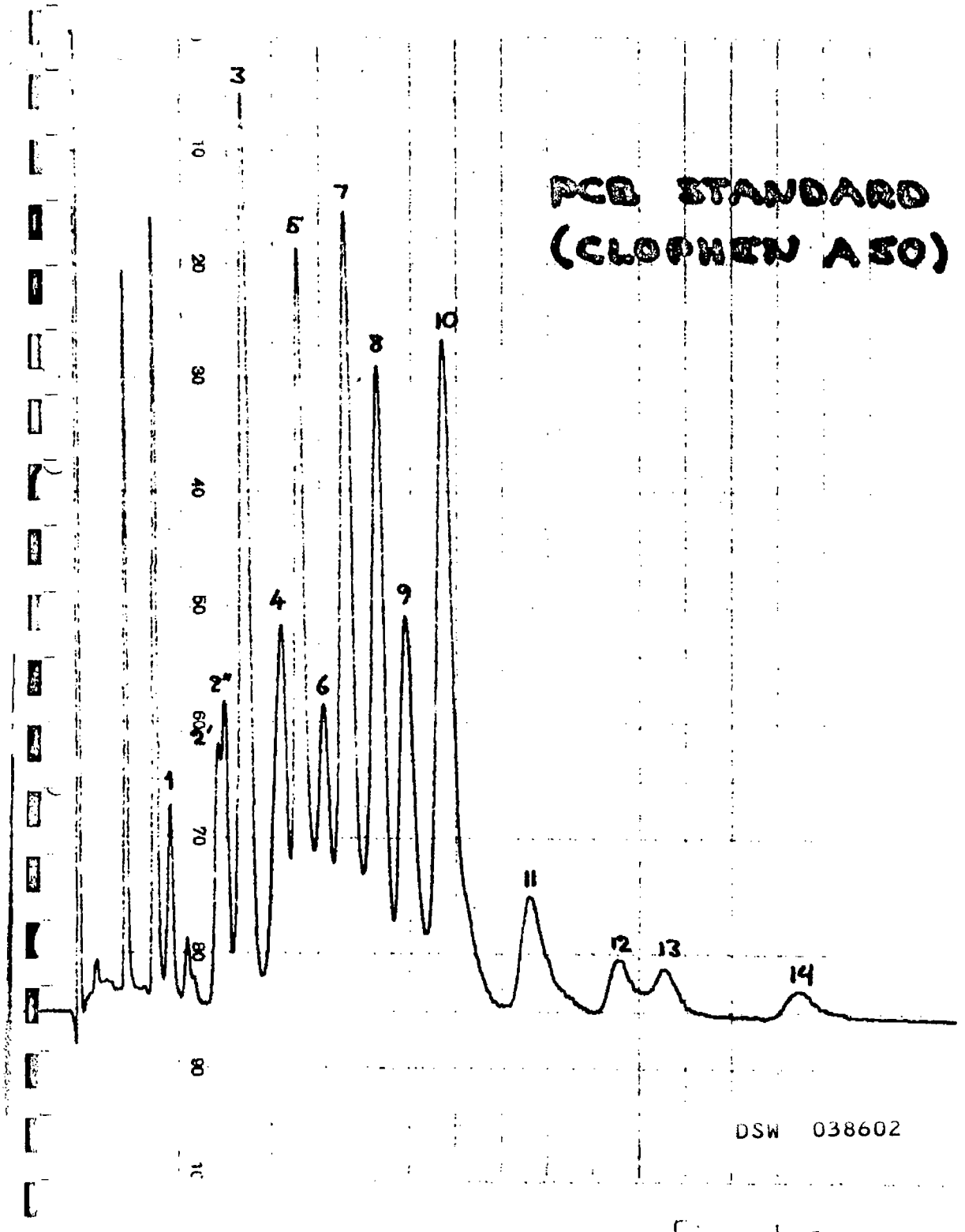

Fig. 1a

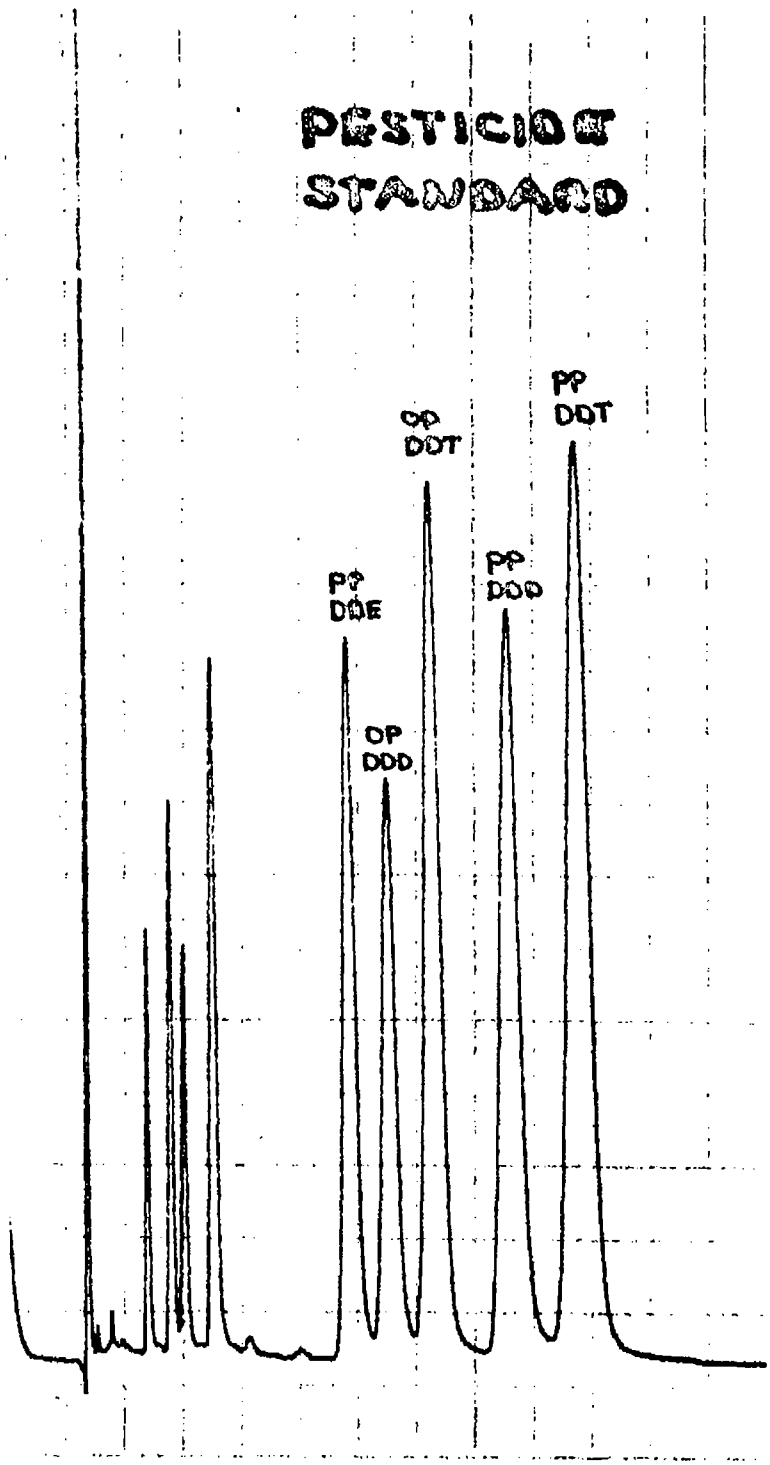

Fig 1b

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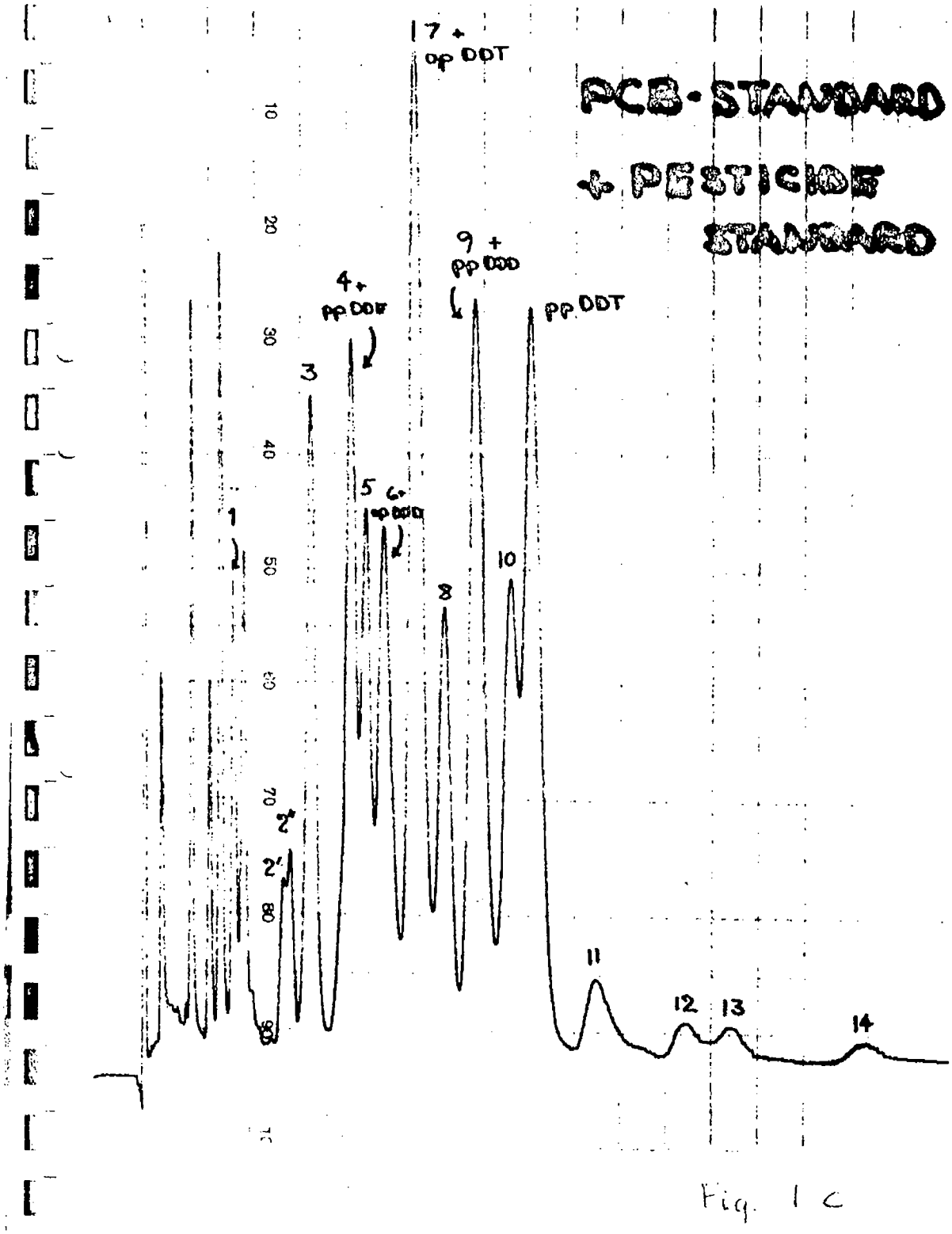

Fig. 1c

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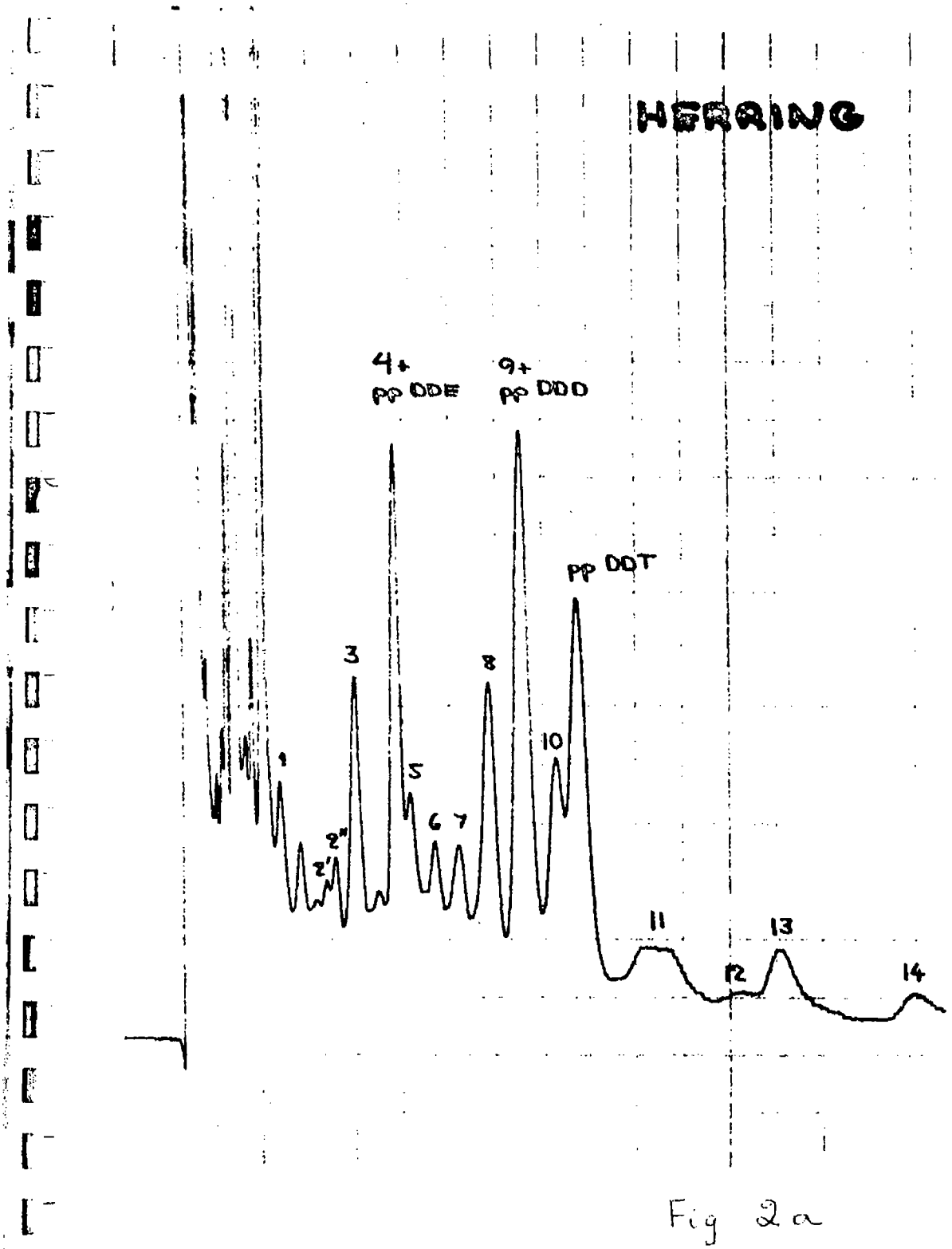

Fig 2a

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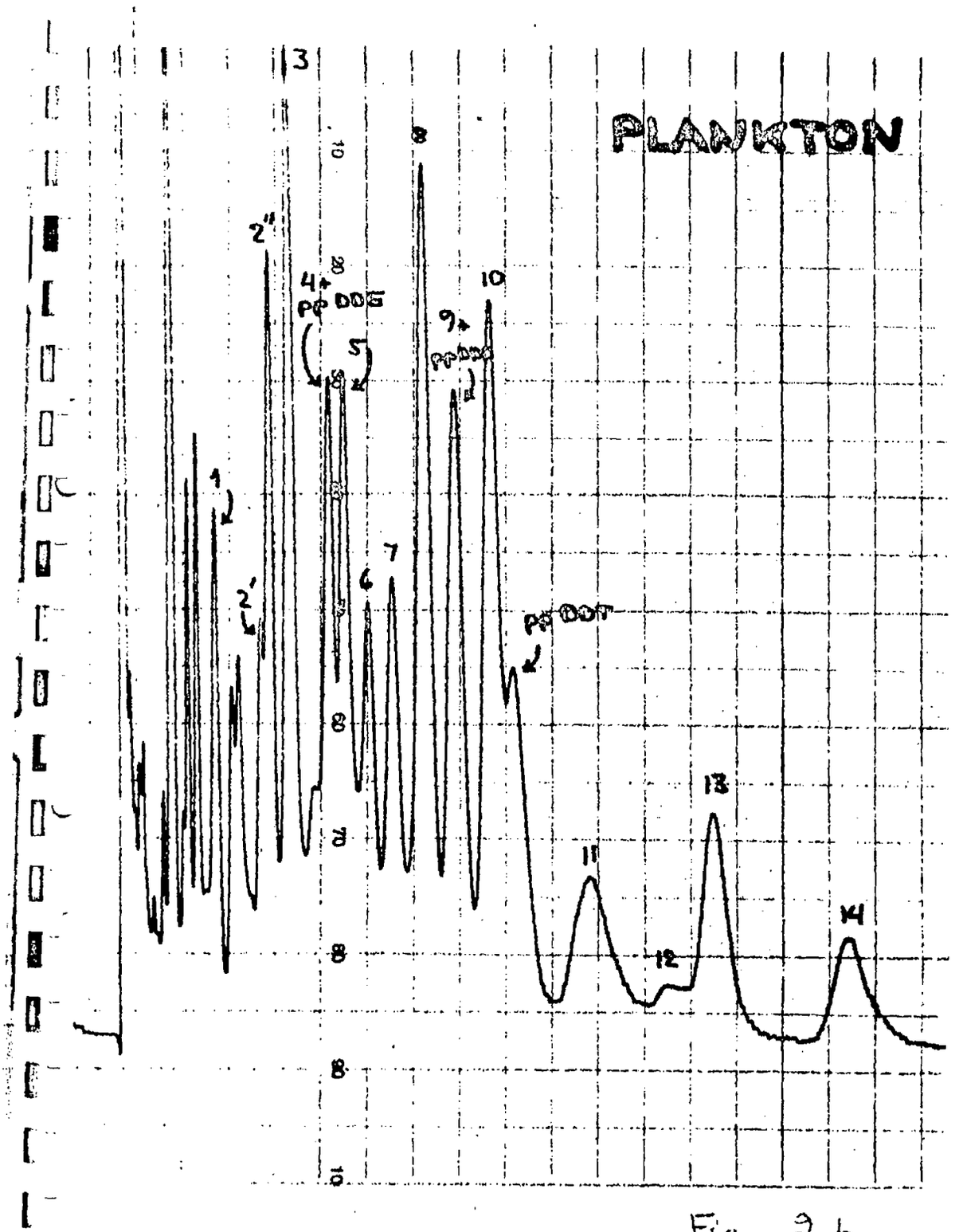

Fig. 2b

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PCB Conference II
Stockholm 1972

Possible health hazards of the polychlorinated biphenyls.

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Since the 1930s it has been known that technical products containing polychlorinated biphenyls (PCB) can cause skin changes, so-called chlor-acne, and inflammatory conditions of the eyelids. Liver damage caused by occupational exposure to PCB has been reported from industry, and recently, in 1968, an extensive epidemic of food poisoning in Japan caused by rice oil contaminated with PCB has been reported, in which neurological signs were observed (1).

To evaluate health hazards in connection with a substance which is distributed in the environment, it is necessary to consider at least three factors:

1. The extent of exposure.
2. The risk of accumulation of the substance or its metabolites in the tissue.
3. The toxicity of the substance of its metabolites.

In the following, I will discuss what is known for PCB in relation to these three factors.

The earlier speakers have already described the common use of PCBs and their spread in the whole eco-system, especially in the aquatic environment. This widespread occurrence causes man to be exposed to PCB by food, water and air pollution. The larger part of the population, in the developed countries at least, is exposed to PCB, and this exposure varies between groups of different occupation and of different food consumption habits.

Is there then any risk for accumulation of PCB or its metabolites in the human tissue? The answer is undoubtedly yes. PCB has

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been identified in human fat in several western countries. Concentrations around 6 ppm have been reported in human fat tissue from West Germany (2), and around 1 ppm in 45% of an examined population in USA (3). Similar figures have been reported from Norweigan autopsy material (4), in which values ranging from 0-5 ppm were found in brain tissues. Two cases in USA have been found to have 200 and 600 ppm in fat tissue (5). Analysis of human breast milk has shown mean concentrations of around 0.1 ppm in West Germany (2). Westöö and Norén (6) in Sweden conducted similar investigations and found a mean concentration in the Stockholm area of about 0.025 ppm. Their investigation was conducted during the period between 1967-1972, and from their results it cannot be excluded that there has been a continuous increase of the concentration of PCB in mother's milk during this period in the Stockholm area. In Japan, not less than a thousand persons were poisoned by PCB contaminated rice oil in 1968 (7). In several of these cases with chronic disease, high concentrations of PCB were found in the tissue eight months after exposure had ceased.

Available data thus strongly indicate a risk of accumulation of PCB in human tissue on prolonged exposure. Present data also give reasons to believe that there is a major difference between different chlorinated biphenyls in this respect. Those that have been identified in human fat are generally the more highly chlorinated ones, pentachlorobiphenyls and up to decachlorobiphenyls (5), while the less chlorinated biphenyls have not been shown to have the same tendency for accumulation and retention in human tissue.

The tendency of PCB to be retained on prolonged exposure does not in itself imply a health hazard. A health hazard will not occur until the concentrations of PCB in critical organs or critical tissue have risen to a toxic level. Our knowledge about the toxicity of the PCBs is very limited and uncertain. Most data concerning the toxicity of PCB is derived from experiments and clinical observations after exposure to technical products of undefined composition with regard to their content of different PCBs as well as to possible impurities originating during manufacture. Vos et al (8) has shown that at least two technical products contain chlorinated dibenzo-

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furans, compounds of high toxicity which cause signs similar to those described with so called PCB intoxication, as for instance liver damage and skin manifestations. It has not been possible from the literature to find out whether Kanechlor, the technical preparation that caused the Japanese epidemic, also contains chlorinated dibenzofurans. However, the fact that this product also gave rise to an epidemic of chick oedema disease in Japan (7) indicates that this may have been the case. So far, no studies on the toxicology of a single defined PCB have been published in the literature. Judged from published observations on the toxicity of technical PCB preparations in mammals and clinical observations from the Japanese poison epidemic, and reported cases of occupational disease caused by PCB containing products, the following effects may arise if a sufficient dose of PCB is retained.

1. Induction and stimulation of liver microsomes. Experimental observations indicate that higher chlorinated biphenyls are more effective than lower (9).
2. Liver damage (7).
3. Interference in fat and steriod metabolism with an increase in triglycerides in plasma, and excretion of 17-ketosteroids in urine (10).
4. Interference in porphyrin metabolism with an increased excretion of porphyrins (11).
5. Acneiform dermatitis (7).
6. Changes in the conduction velocity of peripheral nerves and interference in the cerebral cortical function (1).
7. Foeto-toxic effects causing foetal death or congenital abnormalities as well as retarded development (12).
8. Immunodepression (13).

It cannot be excluded that the PCB products used in experimental studies, and those to which cases of poisoning have been exposed, have contained impurities like chlorinated benzofurans. Thus, some of the above listed effects may, partly or entirely, have been caused by such impurities.

Epidemiological investigations in Japan indicate that the dose of Kanechlor in affected persons was an average around 2 g, and

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varied from 0.5 g and upwards (7). Other data, elucidating dose-response relation for toxic effects caused by PCBs in man, are not available. It has to be pointed out, however, that the Japanese figures relate to Kanechlor and do not permit conclusions about different types of chlorinated biphenyls and the occurrence of toxic impurities in Kanechlor cannot be excluded.

CONCLUSION

The conclusion is that it is today impossible to estimate the health hazards to man due to long term exposure to PCB, as we lack knowledge about their toxicity. There is an appreciable exposure to these compounds in our communities and there is an evident tendency to accumulation of chlorinated biphenyls in human tissue. It cannot be excluded that the present spread of PCB in the environment can involve significant health hazards in the long rung, and it cannot be excluded that there is a continuous increase of exposure. It is questionable whether steady state between pollution and distribution in the eco-system is obtained at present.

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PCB Conference II
Stockholm 1972

MONITORING PCB IN WATER AND WILDLIFE

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INTRODUCTION

Although polychlorinated biphenyls (PCB) have been manufactured for various purposes for the past forty years, it was not until 1966 that Jensen identified them in a variety of environmental samples, using the gas chromatographic technique employed since 1962 for the detection and measurement of the persistent organochlorine pesticides such as DDT (and its metabolites) and dieldrin. Confirmation of the identity of the PCB residues was made by mass spectrometry, which at the same time indicated the presence of a larger number of polychlorinated biphenyls than was suggested by the number of gas chromatographic peaks.

In the analysis of wildlife samples for organochlorine pesticides since 1962, several workers had recorded the presence of a number of unidentifiable GLC peaks. Jensen's discovery of the presence of PCB residues, while assisting in the determination of a further group of detectable contaminants, made it evident that some of the earlier analytical results reported in the literature, particularly for pp'-DDT and pp'-TDE, were probably erroneous. Subsequently techniques have been developed to enable a reasonable degree of separation of PCB from many organochlorine pesticides to be achieved before GLC analysis, but the PCB residues must still be estimated as a group at present.

Polychlorinated biphenyls can be used for a wide variety of purposes, and the mixtures of isomers produced by the various manufacturers may range from those containing about 20% by weight of chlorine, averaging about one chlorine atom per molecule, to some containing about 70% of chlorine, averaging almost nine chlorine atoms per molecule. A total of 209 chlorinated biphenyls, containing from one to ten chlorine atoms, is theoretically possible, and it has been shown (Sissons and Welti, 1971) that one particular 54% chlorinated mixture contains at least fifty different isomers. Few of these have been prepared individually, and consequently for routine analysis of environmental

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samples the commercially-available mixtures must be used as reference standards. Some degradation of the lesser-chlorinated members of the series probably occurs in the environment, and in consequence the pattern of GLC peaks obtained from wildlife samples differs from that of the commercial mixtures. The estimation of total PCB content and toxicity of the wildlife residues are therefore only approximate at the present time.

In view of the persistence of the higher polychlorinated biphenyls, their presence at all environmental levels, their potential interference in the analysis for organochlorine pesticides, and their possible toxicity to various forms of life, considerable attention has been given in recent years to the problem of their world-wide distribution. Most studies have involved fish and birds, but in view of the possibility that global monitoring programmes for pollutants such as organochlorines may be established in the near future some consideration must be given to the types of environmental samples most suitable for the purpose of monitoring.

The Organisation for Economic Co-operation and Development(OECD) has for several years sponsored voluntary international studies of the problem of sampling and analysing wildlife specimens for organochlorine residues, and this paper summarises the results of the first six years of investigation, and indicates the difficulties involved both in the analysis of the samples and in the selection and acquisition of the necessary sample material. It has been possible to compare the efficiency of analysis in different countries, and to assess the importance of analytical errors as compared with the variations between individuals in populations of certain species. The occurrence of PCB concentrations in environmental media as opposed to living organisms is also discussed, media samples sometimes being suggested in preference to wildlife samples for monitoring purposes.

ANALYTICAL METHODS

PCBs are extracted and analysed by the same techniques as are employed for organochlorine pesticides, and appear to be recovered from water and tissue samples with the same efficiency, but must be separated from the majority of pesticides before GLC analysis to avoid interference. Separation is usually made using silica column chromatography,

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the PCB compounds being eluted with p,p'-DDE (Holden and Marsden, 1969), although Armour and Burke (1970) achieved a degree of separation of the PCBs from DDE with a more active type of silica. Several methods have been proposed for the determination of the total PCB content from the gas chromatographic trace, but a recent OECD study (Holden, in preparation) has shown that a considerable measure of agreement can be obtained among analysts using different methods of estimation.

Peak height is usually measured although peak area, measured by electronic integration or mechanical devices, is sometimes used; 4 to 6 GLC peaks are commonly involved. The estimation of PCB concentration is influenced to some extent by the choice of a suitable reference standard, which is usually arbitrary in view of the difference in proportions of the various PCB peaks, as between environmental samples and commercial products. Nevertheless, in the recent OECD study, eight analysts using different methods of estimation but similar 60% chlorinated reference mixtures obtained results on a cormorant sample which showed a coefficient of variation of ±14%, a value no greater than was obtained for some individual pesticides in another exchange sample used in the same OECD study. An alternative method of assessment, not yet widely used, is the complete chlorination of all PCB residues found to the deca-chloro compound, giving a single GLC peak which is compared with the same peak produced from any PCB formulation, or a deca-chloro-biphenyl standard.

Where low-level concentrations are being determined, as in water or plankton samples, great care is necessary to avoid contamination from laboratory or other sources which may give GLC peaks in positions similar to those from the samples. Such contamination, insignificant in the analysis of samples containing high concentrations of PCBs, can be derived from plastics (particularly PVC), oil used in air compressors, human skin (if not adequately washed beforehand), internal seals of metal containers, and in the solvents or adsorbents used in analysis. All solvents must be purified by distillation, and all adsorbents or drying media (e.g. alumina, silica, sodium sulphate) must be calcined or solvent-washed before use. Blank runs through the complete analytical procedure must be made frequently to check for possible contamination.

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PCB LEVELS IN THE ENVIRONMENT

(a) Air and Rain No reference has been found in the literature to concentrations of PCB in gaseous form in air, and it is quite possible that atmospheric PCB is associated with particulate matter (dust) or water droplets. In the sampling of air, such forms would be removed by filters and analysed as particulate matter. Risebrough et al (1968)[a] found 1 to 64 ppb of organochlorines in dust from air over Barbados, but did not positively identify PCB. Analysis of airborne dust off the California coast did not reveal the presence of PCB, and the deduction was made that PCB do not adsorb to particulate matter.

In the sampling and analyses of atmospheric precipitation for organochlorines, only the common pesticides ∝- and Y-BHC, dieldrin, pp' DDT and its metabolites pp'-TDE and pp'-DDE have been reported in detail. Wheatley and Hardman (1965) and Tarrant and Tatton (1968) found these residues in rain in the United Kingdom, but the latter also noted the presence of other residues believed to be PCB, without quantifying them. Their existence was confirmed in further rain samples collected in London (Annual Report of the Laboratory of the Government Chemist, 1970), with an approximate (TLC) estimate of their concentration as 50-100ng/l.

Rain samples have been collected and analysed from four different sites in Scotland during 1972, and preliminary results indicate that PCB levels are in the range 20-40ng/l, with total DDT levels of 5-20ng/l. These samples are taken in all-glass equipment, protected from birds, the aerial debris (dust, pollen, etc.) being retained separately on pre-washed glass wool filters which are also analysed. Several hundred millilitres of rain must be collected, the sampling periods being irregular according to the prevailing weather conditions. The concentrations of organochlorines found in the samples are nevertheless much higher than in river and stream waters in Scotland. Furthermore, there is so far no evidence that the residues are transported from industrial areas of Scotland by prevailing winds and it seems likely that the contamination is of more distant origin, or perhaps reflects a general level of atmospheric contamination. In this form of sampling, however, it is impossible to exclude the possibility of transient local contamination, whether from

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the excreta of flying birds, from insects, or passing transport, and such events should not be ignored.

(b) Fresh Waters A few references to PCB concentrations in rivers have appeared in the literature, although these generally refer to local contamination. In the Milwaukee River, U.S.A., 20-260ppt of Aroclor 1260 and 80-2070ppb of Aroclor 1242 were detected (Veith and Lee, 1971). In Lake Biwa, Japan, 100-1000ppt of PCB were found (Isono, 1971). Ahling & Jensen (1970) found 0.33 and 0.50ppt in natural waters in Sweden, but particulate material contained 1350ppt. The sediments in Ontario (Canada) rivers have been found to contain up to 0.6p.p.m. (Berg et al, 1972), and in several U.S. rivers up to 3.2ppm (Yates et al, 1972). The latter also found 0.1-4ppb in unfiltered river waters from the United States. The Michigan Water Resources Commission has reported 10-2900ppt in 30 tributary streams to the Great Lakes (Anon, 1972).

Analyses of Scottish streams and rivers for organochlorines in 1971 and 1972 have shown that, even in streams with no evidence of industrial or gross sewage pollution, the PCB concentrations are in the range 2-20ppt in unfiltered samples. The concentrations of organochlorine pesticides found were 0.1-5.1ppt dieldrin, 0.3-5.3ppt DDE, 0.3-6.4ppt TDE and 0.2-5.7ppt DDT, excluding badly-polluted rivers. These residues, with the possible exception of dieldrin (used for about ten years in sheep dips throughout Scotland until 1966), are believed to result from atmospheric, rather than terrestrial contamination. The PCB estimations were made on the basis of fewer peaks than is customary for tissue samples, and further examination is likely to reduce the values quoted above.

(c) Sea Water Analysis of a sea-water sample from the Firth of Clyde, West Scotland, an area which receives about one million tons of sewage sludge per annum, showed a concentration of 8ppt. Previous investigations (Holden, 1970) had shown that about one ton of PCBs were probably contained in the annual discharge of sludge but during 1972 the source from which the PCB entered the sewer was removed. Sea-water samples from the Firth of Tay, East Scotland, contained 5-20ppt, the higher values

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being in the vicinity of the city of Dundee. Much of this may have been in particulate matter.

(d) Freshwater Fish The levels of PCB in freshwater fish have been reported from only a few areas, partly due to the fact that pesticide concentrations in rivers and streams in agricultural areas are often greatly in excess of PCB levels, but also, as in Scotland, because terrestrial sources of direct contamination are often non-existent. The PCB problem has so frequently been related to the marine environment that their presence in fresh waters may have been overlooked, yet discharges of industrial wastes or crude sewage to rivers would be expected to result in significant PCB accumulation in the biomass. Possibly the fact that such rivers, if heavily polluted, may contain no fish has produced this situation, as fish are commonly used as the monitoring species for such pollutants.

Yet fish, like many other aquatic species, can concentrate PCB by at least three orders of magnitude, and it would be surprising if PCB residues were not detectable in the fish of the more polluted rivers and lakes. Zitko and Choi (1971) reported a concentration of 14.6 ppm in a coho salmon (Oncorhynchus kisutch) from Lake Michigan. Other fish from Lake Michigan analysed by Veith and Lee (1970) contained 15-25ppm. Fish from the Great Miami River (Kroner, 1971) had up to 150ppm, the PCB being discharged by paper mills. Zitko (1971) found 0.36-1.01ppm in American eel (Anguilla rostrata) from New Brunswick (Canada) rivers and, as there was no evidence of industrial or sewage pollution in some of these rivers, Zitko considered atmospheric fallout to be a possible source.

(e) Freshwater Invertebrates Invertebrates are less often studied than fish, but Södergren et al (1972) used the amphipod Gammarus pulex as an indicator organism for both DDT and PCB in streams in southern Sweden. There was evidence of a seasonal variation, with higher residue levels in August than in April, the PCB concentration being 0.002-0.011ppm. A number of other invertebrates were also examined, but PCB concentrations never exceeded 0.020ppm, total DDT being up to 0.046ppm.

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(f) Marine Fish and Shellfish. Zitko and Choi (1971) summarised the analytical data reported for marine fish, the concentrations of PCB in the presumably unpolluted areas being less than 0.1ppm. In areas known to be polluted, such as the California coast, the Baltic and the Bay of Fundy, values up to 1.0ppm have been found. In a heavily polluted area such as Escambia Bay, Florida, concentrations from 4.5 to 20ppm were detected. Tissues containing a high proportion of lipids were more heavily contaminated.

In a survey of the organochlorine content of commercial marine fish species from waters around the United Kingdom, (Portmann and Holden, unpublished), PCB concentrations in muscle tissue ranged from less than 0.1ppm in fish such as cod (Gadus morhua) up to 1.5ppm in plaice (Pleuronectes platessa) and mackerel (Scomber scombrus) in the polluted waters of the Firth of Clyde. Fish liver reflected contamination of the environment even more markedly, with concentrations in cod liver ranging from 0.4 to 43ppm. Expressed in terms of extractable lipid, PCB levels may range from 1ppm in unpolluted to over 300ppm in polluted areas.

The shellfish most commonly used for pollution studies have been molluscs such as oysters and mussels. However, these species seem to be able to rid themselves of contaminants such as pesticides relatively quickly, and the concentrations found may not always reflect the external conditions at the time of sampling. Nevertheless, persistent pollution in estuaries will give rise to abnormally high residue levels in mussels as found in surveys made in Scotland, using Mytilus edulis. Specimens in the Firth of Clyde contained up to 3.0ppm, while in clean waters the concentrations of 0.1-0.2ppm were more usual. The overall range, however, is not very large.

(g) Marine Plankton Marine zooplankton, with a significant lipid content, might be expected to reflect PCB levels in the surrounding aqueous medium, but two major difficulties arise in their use for monitoring. Zooplankton samples usually vary in species composition, and this may result in a between-sample variation in PCB content by virtue of the different lipid contents of the various species. Analyses based on lipid content might, however, reduce the influence of species composition. A more serious problem arises from the possibility of collecting in the plankton net

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such extraneous material as refuse from ships, and tar or oil, which are found almost everywhere on the ocean surface. Nets used for plankton should be closed when entering or leaving the water surface. As oil is an excellent solvent for PCB, even a small amount may produce a significant enhancement of PCB concentrations in plankton samples. Paint, hydraulic fluid or lubricating oil from ships could also be sources of PCB.

Despite these potential errors, analyses of mixed zooplankton samples in the eastern Atlantic and Firth of Clyde have shown a consistent pattern, the latter containing higher concentrations, as expected from the local PCB contamination. The samples from the north-east Atlantic contained 0.01-0.11ppm wet weight (0.1 - 4.2ppm in lipid), while samples from the Firth of Clyde, including some unpolluted areas, contained 0.04 - 2.20ppm wet weight (1.1 - 20.0ppm in lipid). Extremely high PCB concentrations, expressed in terms of extractable lipid, were obtained from plankton (more correctly seston) samples in the Bristol Channel, S.W. England, where the sediment is continually disturbed by tidal movements. The area receives a considerable amount of industrial waste, and PCB values of up to 7000ppm in lipid were calculated seston. Such a situation would be unlikely in the open ocean. Recent analyses of plankton from the Clyde suggest that species composition may have little influence on the PCB levels in zooplankton samples.

(h) Birds Both terrestrial and aquatic species of birds may accumulate high PCB concentrations, but the piscivorous species, particularly in the marine environment, generally contain most. Risebrough et al (1968) recorded the results of analyses of both DDT group and PCB residues from a wide range of avian species in North America. Some species contained less than 0.01ppm in muscle tissue, but values between 0.1 and 1.0ppm were common, and in species feeding on fish in polluted areas concentrations between 10 and 100ppm were common. Zitko and Choi (1971) summarised the analyses of birds from a number of literature references, and in a few instances, such as in the Stockholm Archipelago, values between 100 and 10,000ppm PCB were found. Such concentrations would seem to be potentially, if not actually, toxic to the birds. The eggs of several species in contaminated areas have also been found to contain high levels of PCB, sometimes 10 - 100ppm.

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The range of PCB residues in birds from various locations is thus extremely wide, from 0.01 to 10,000ppm, and much larger than is found in fish, for example. In this respect, birds would appear to be most appropriate organisms for monitoring the distribution of PCB in the environment, although other considerations must qualify this judgement, as discussed later.

(i) Marine Mammals Holden, (1972, and 1973 in press) has described the analysis of seal and porpoise fat (blubber) for organochlorine residues, and the variation in PCB concentrations found in seals from different parts of the world. Concentrations in extractable lipid range from 1ppm in the Arctic to 250ppm on the west coast of the United Kingdom, but in starving specimens, which have metabolised most of their fat reserves, up to 2800ppm has been found. The increase in concentrations in lipid, as the result of starvation stress and reduction of body fat reserves, is a complicating factor in the use of residue concentrations in organisms for assessing the extent of local environmental contamination.

MONITORING PCB IN THE ENVIRONMENT

The above summary has indicated the range of concentrations of PCB to be found in the environment, but before any selection of species appropriate for monitoring is made several aspects of the problem of environmental contamination must be considered. The contaminant in question may enter an organism from the external medium, such as air or water, or from sediments in the case of an aquatic filter-feeder, and thus the media might be appropriate for sampling and analysis. However, contamination of water or air might fluctuate appreciably with time, and organisms, in which the fluctuations would be reduced, could reflect the general situation more effectively, while at the same time providing higher concentrations for easier determination. As uptake from food is an alternative, and often the only, source of contamination in most species, the concentration in the external medium may not be directly relevant.

Species of which the individuals move over considerable distances, either for feeding or on migration, may give residue information which is difficult to interpret, as the residues may not have been ingested in the area in which the specimens were

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obtained. Thus adult migratory birds are not appropriate monitoring organisms. For the same reason, marine fish, which may move from estuarine to oceanic waters, could also be unsuitable. Freshwater fish which remain in a lake over a complete life-span, or invertebrates, which move over relatively short distances, can on the other hand provide information relevant only to their local environment.

A further important consideration in the choice of species for monitoring is that sufficient individuals must be available for sampling at the required time, without having a significant influence on the total population. As residue levels often increase with age, and may also differ between the sexes, the specimens may have to be selected both for age and sex, thus increasing the problem of sampling. Furthermore, there is always a natural and sometimes considerable variation among individuals within a population, even when of one sex and age. In consequence, to obtain a reasonably accurate estimate of the mean level of contamination in any one population, a large number of individuals may be required, creating problems both in sampling and analysis.

At this point, it is worth considering the purpose for which a monitoring programme may be designed. Initially it is intended that the level of contamination in several areas will be determined, so that the more heavily contaminated areas can be identified, and the sources of pollution controlled. Yet, unless these are already known or suspected, and sampling areas chosen accordingly, a general monitoring programme is unlikely to reveal such "hot-spots". On the other hand, contamination over large areas, or due to aerial fallout from distant sources, may be detected in a general survey.

The information obtained must, however, be sufficiently accurate to establish statistically that levels of contamination appearing to be high are in fact high, by comparison with those in other areas. Similarly, it should be possible for changes in the contamination level from year to year to be detected with appropriate statistical significance. Such information is of value in assessing the consequences of government or industrial action which has been taken to reduce pollution. The accuracy of the estimates of contamination levels depends in part on the precision of the analyses, which in turn is dependent on the experience and efficiency of the analysts.

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In large-scale monitoring programmes many analysts will be involved, but an adequate assessment of their agreement can be obtained by the use of exchange samples.

Finally, the acquisition of analytical data is not the ultimate purpose of monitoring. The underlying fear is that contamination levels may be sufficient to have adverse biological effects at one or more points in an eco-system, but as yet adequate evidence of such effects has been established in very few instances. It is essential that the concentrations in various species which are capable of causing adverse effects should be determined, whether the effects are for example, reduced photosynthetic activity in algae, the failure to reproduce in invertebrates, abnormal behaviour in vertebrates, or mortality in any species.

OECD STUDIES OF MONITORING

The Organisation for Economic Co-operation and Development (OECD), in a series of international collaborative studies on the monitoring of organochlorine residues in the environment, has attempted to examine some of the problems referred to in the preceding paragraphs.

In the second programme (Holden, 1970b), the ability of analysts in several countries to obtain the same results, both qualitatively and quantitatively, in the analysis of exchange samples was measured. At that time (1968-69), the variation between analysts increased with the difficulty of processing the samples, and for the only residues in which there was general agreement on identity (dieldrin, DDE, TDE and DDT), the coefficient of variation was ±25 to ±60%. In the third programme (Holden, in preparation) a substantial improvement was noted for the period 1969-71, the coefficient of variation for six pesticide residues and PCBs in samples requiring full processing being ±10 to ±18% among 17 laboratories.

The question of the number of individuals of a population required to provide a mean value of sufficient precision for detecting differences between populations, or changes from year to year, was also examined in the second programme. In both starling and pike populations (with no selection for age or sex), fifty individuals would have been required to detect a 25% difference between means, but ten would have been

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sufficient to detect a 75% difference. The main reason for such low precision was that the residues in the individuals were usually skewly distributed, but this may have been due to the variation in age within samples.

The third study programme included the analysis of PCB residues in addition to organochlorine pesticide residues in selected wildlife species, and samples were taken from uncontaminated and contaminated areas. The results of this programme have provided information on the extent of PCB contamination, and also on the difficulty of identifying the contaminated areas.

It must be borne in mind that, in common with all other PCB analyses at the present time, the values obtained are estimates of the total PCB concentration expressed in terms of a commercial mixture of specified chlorine content. The proportions of the various isomers and homologues present in the samples usually differ from those in the commercial mixtures, and no information on the concentrations of individual compounds is afforded by the analysis. The assessment of the potential toxicity of the residues detected is thus rendered difficult, and any such estimate must be regarded with caution.

Ten countries sampled and analysed the mussel (Mytilus edulis) in coastal waters, both in areas considered to be uncontaminated and in other areas known to be polluted. The assessment of pollution varies considerably, some polluted areas giving PCB concentration in mussels as low as 0.02mg/kg, and one unpolluted area giving 0.67mg/kg. Areas with varying degrees of pollution gave values of 0.02 - 1.7mg/kg. A similar situation arose with the samples of herring (Clupea harengus) analysed by eight countries. Those from areas classed as unpolluted had PCB concentrations in muscle tissue ranging from 0.10 to 0.54mg/kg, but the range in fish from polluted areas was 0.19 to 3.2mg/kg.

Freshwater fish show less evidence of PCB contamination. The pike (Esox lucius) sampled in six countries usually contained little PCB even in polluted waters, the values generally being less than 0.1mg/kg. Yet where serious pollution by PCB exists the concentrations can be much higher, as in the samples analysed by the Netherlands, with PCB levels of 0.47 - 0.95mg/kg.

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The eggs of fish-eating birds can demonstrate the accumulation of organochlorine residues from their food very effectively. Eggs of the heron Ardea cinerea were analysed by the United Kingdom and the Netherlands. In samples from remote areas of the United Kingdom concentrations below 0.5mg/kg were found in the eggs, with up to 7.3mg/kg in a very polluted area. In the Netherlands the concentrations in polluted areas were up to ten times greater, 33 - 74mg/kg. This ability to concentrate residues in eggs was also shown by the samples from two common tern (Sterna hirundo) colonies in Canada. The relatively uncontaminated colony gave .2 - 4mg/kg PCB but that in a polluted area contained a mean of 149mg/kg in eggs.

DISCUSSION

The third OECD programme has indicated the difficulties of identifying areas polluted by organochlorines, and in particular PCB, by evidence other than chemical analysis. Consequently the identification of local sources of PCB pollution by the analysis of indicator or monitoring species would entail a very large and detailed sampling and analytical programme involving numerous sites. On the other hand, the detection of more general pollution over large areas, if such pollution exists, should be disclosed by a pattern of fewer sampling sites at which an appropriate species could be used for monitoring on a regular basis. Ideally, this species should accumulate the contaminants to such an extent that there is a considerable difference between PCB content of uncontaminated and polluted specimens. The species should therefore be relatively resistant to the effects of the pollutant.

Referring to the information provided by the second OECD study, concentrations of PCB in mussels showed an overall range of about 1:100, with only a 1:30 range for herring. The eggs of herons showed at least a range of 1:150, and an even greater range might be revealed by further samples. A heron in the Stockholm Archipelago was found by Jensen et al (1969) to contain 9,400mg/kg in muscle, which suggests that eggs from such birds could also contain exceptionally high levels.

While eggs may be particularly useful in revealing areas of pollution, the numbers available for analysis could be limited. On the other hand species such as mussels or

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herring can usually be collected in quantity. The greater precision of the estimation of the mean level of contamination of a population of mussels, herring or similarly abundant species could therefore be as important in the choice of a monitoring species as the wider range of contamination levels likely to occur in the eggs of piscivorous birds.

A further restriction is placed on sampling by the habitat of the species. Mussels can only be collected from coastal waters, for example, and seals, in which extremes in contamination level have also been demonstrated (Holdom, 1972) are limited in their distribution. As many seal colonies are protected, it may be difficult to acquire more than a few specimens for analysis. The major problem which arises in the selection of an appropriate monitoring species for global surveillance, however, is that very few occur in all areas of the world, although an ecologically equivalent species can often extend the range. Species which seem ideal from many points of view (adequate numbers, ease of sex and age determination, and of sample) may be limited to the northern hemisphere, or to one continent. Marine species are sometimes more widespread, but clearly cannot reflect contamination in terrestrial or freshwater environments, where pollution may originate.

The OECD studies have enabled some assessment to be made of analytical accuracy, of the ability to obtain samples of chosen species in the required numbers (or of the attendant difficulties), and of the ranges of various residues which exist in polluted and unpolluted areas. They have also shown the difficulty of positively identifying such areas prior to performing the analyses. In particular, the choice of species which would be appropriate for global monitoring has been under constant discussion, and no species has yet been proposed which meets all the requirements for such a purpose. It is likely that a wide variety of species must eventually be proposed, the assessment of the extent of pollution being based on the results from several species in each area, rather than on any particular species.

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PCB in mammals and birds in the Netherlands.

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In the Netherlands PCB's were observed for the first time in sandwich terns (Sterna sandvicensis) and eider ducks (Somateria mollissima) during a study of a case of bird mortality which was caused by industrial pollution with pesticides (mainly dieldrin and telodrin) (Koeman et al., 1967; 1969; 1972a; Koeman, 1971; Koeman and van Genderen, 1970; Swennen, 1972).

Since then it was shown in various studies that PCB's are present in many species both in the aquatic and terrestrial environment. In the present paper the main results will be summarized. Some data will be presented concerning an experimental study with small groups of cormorants (Phalacrocorax carbo) and herons (Ardea cinerea). The paragraph on seals, porpoises and dolphins includes data on dolphins which were obtained from New Zealand and Dutch Guiana.

For sample clean-up the techniques devised by Holden and Marsden (1969) and Armour and Burke (1970) were applied. The final detection was based on gaschromatography. For some species analysed, tissue extracts could be submitted to mass-spectrometric analysis by using a GC/MS combination (Koeman et al. 1969; ten Noever de Brauw and Koeman, 1973). A description of the methods currently in use for

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wildlife samples in the Netherlands was published by de Vos (1972). The quantitative estimation of PCB residues was carried out by peak-height comparisons using standard solutions containing a 60% technical PCB-mixture (Phénoclor DP 6).

1. PCB in birds of prey and owls.

In recent years various studies on birds of prey and owls in relation to pesticide contamination were carried out by the Research Institute for Nature management (Fuchs et al., 1971, 1972). The main purpose of these studies was to measure the change in residue levels following the official bans and restrictions on seed dressing applications on aldrin, dieldrin, heptachlor and methylmercury compounds which were enforced in 1968 and 1969. In the same studies the residue levels of PCB were measured. The average levels found are presented in table 1 (data from Fuchs et al. 1972). The levels found appeared to be particularly high in the long-eared owl (Asio otus) and the barn owl (Tyto alba). In one long-eared owl a liver level of 590 ppm was recorded. Among the birds of prey the levels were highest in the kestrel (Falco tinnunculus).

Table 1. Residue levels of PCB in the livers of birds of prey and owls. Mean residue levels are compared during three subsequent winters (data from Fuchs et al., 1972).

species	number of individuals in sample			PCB concentration (ppm wet weight)		
	68/69	69/70	70/71	68/69	69/70	70/71
buzzard	21	14	9	13.68	11.64	5.31
sparrow-hawk	5	11	16	9.20	5.12	6.81
kestrel	5	13	7	21.40	18.66	44.13
barn owl	5	5	3	43.82	28.80	3.02
long-eared owl	3	9	8	191.00	84.82	6.85

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3.3. PCB in marine birds.

Data on PCB levels were obtained for eider ducks and sandwich terns. The levels found in liver, brain and blood samples of eider ducks are presented in table 2. From the pesticide levels found in the tissues of the birds found dead or dying it could be concluded that poisoning by toledrin and dieldrin very probably was the main cause of death. Mortality only comprised female birds and occurred predominantly at the end of the incubation period. Female eiders consume little or no food when breeding. Apparently the chlorinated hydrocarbon compounds were mobilised from fat and other tissues in the course of incubation. In 1966 it could be demonstrated that the blood levels rose steeply (about twentyfold increase) (Koeman and van Genderen, 1970; Koeman, 1971). For this reason it must be realised that the PCB levels for dead and dying birds in table 2 also reflect the tissue distribution pattern at the end of the incubation period.

Table 2. Residue levels of PCB in tissues of eider ducks.

year	history	tissue	n	PCB concentration (ppm wet weight) mean and range
1967	found dead or dying	liver	10	63 (23-96)
		blood	10	24 (16-37)
	shot	liver	5	5.5 (2.1-12)
1968	found dead or dying	liver	9	89 (67-134)
		brain	4	55 (48-62)
	shot	liver	3	5.6 (3.9-6.5)

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PCB residues in eggs of eider ducks and sandwich terns are given in table 3.

Table 3. Residue levels of PCB in eggs of eider ducks and sandwich terns.

species	year	n	PCB concentration (ppm wet weight) mean and range
eider duck	1968	10	16 (12-21)
	1970	5	5.8 (5.3-6.5)
sandwich tern	1967	10	4.5 (1.9-6.5)
	1969	15	11 (0.92-31)
	1970	10	7.1 (3.7-15)

2. PCB in cormorants and herons.

PCB levels found in cormorants and herons are summarized in table 4.

Table 4. Residue levels of PCB in tissues of cormorants and herons.

year	species and history	tissue	n	PCB concentration (ppm wet weight) mean and range or range
1970	cormorants found dead	liver	6	320 (93-470)
		total body	6	29-460
		brain[1)]	6	190
1970	cormorant nestlings	liver	6	0.38 (0.078-0.61)
		total body	6	2.4 (1.9-3.2)
		brain[1)]	6	0.69
1970/1971	herons	liver	18	7.7-712

[1)] residue level in mixed homogenates.

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It could be calculated that some of the cormorants found dead contained as much as approximately 1 g of PCB in their bodies. In order to assess the possible toxicological implications of PCB on the cormorant an experimental study was carried out with 5 cormorants which were taken from their nest in the spring of 1971. In the autumn of 1971 all 5 birds were treated with PCB (Clophen A 60). The dose rate at the beginning of the experiment was 100 ppm calculated on an amount of about 250 g fish which was administered to each cormorant every day. In the course of the experiment the dose rate was increased stepwise to 500 ppm after 14 weeks. The main results are given in table 5.

Table 5. Residue levels in tissues of cormorants poisoned with PCB (Clophen A 60) under experimental conditions.

no	sex	total dose (g)	survival time (days)	residue level (ppm wet weight) brain	liver	fat	total body 1)	total body content (g)	% of dose
1	♀	2.3	55	115	210	n.a.[2]	850	1.12	49
2	♂	3.5	75	160	230	n.a.	1820	2.75	79
3	♀	6.7	106	76	245	10,300	2750	4.85	72
4	♀	7.1	110	180	290	20,500	2150	3.34	47
5	♂	9.1	124	120	285	n.a.	2580	4.15	46

1) total body minus bill, feathers, legs and feet.

2) n.a. = not analysed

It appears from the table that the rate of retention of PCB in the body was high and varied from 46 to 79 percent. The actual amount must have been slightly more because several parts of the birds were not included in the total homogenate. Average levels of 130 and 252 ppm PCB were detected in the brains and livers respectively. No correlation was found between the concentrations in these tissues and the time of survival, which suggests that these represent threshold concentrations for the cormorant. Comparing these levels with those

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in the birds found dead (table 4) it seems highly probable that the wild birds had died as a consequence of PCB poisoning. A more detailed discussion on this experiment was given elsewhere (Koeman et al. 1973).

Liver necrosis and hepatic porphyria are a general finding after PCB poisoning in various species of laboratory animals (Vos and Koeman, 1970; Vos and Beems, 1971; Vos et al., 1971). These symptoms were not seen in the cormorant. Hydropericardium and subcutaneous edema, symptoms observed in chickens which were fed the same lot of technical PCB as was given to the cormorants, were not observed in the latter species. These symptoms were due to the presence of chlorinated dibenzofurans in the mixture used (Vos et al., 1970). Apparently the cormorants did no respond to these compounds.
In cormorant eggs a significant correlation was found between shell thickness and the concentrations of PCB, DDE and hexachlorobenzene (HCB). The correlation was not significant for dieldrin (table 6).

Table 6. Residue levels of PCB and some other chlorinated hydrocarbon compounds in 63 cormorant eggs which were divided into 8 groups according to egg shell thickness[1]

egg shell thickness ($mm.10^{-2}$, mean and range)	number of eggs	residue level in ppm (wet weight) PCB	DDE	HCB	dieldrin
47.5(46.4-49.2)	9	40	3.0	0.45	0.53
45.4(44.9-46.1)	9	42	3.2	0.50	0.35
42.2(41.2-44.0)	9	46	4.0	0.31	0.28
40.4(39.9-41.0)	9	85	5.8	0.53	0.48
38.5(37.4-39.4)	9	50	4.4	0.63	0.44
36.9(36.2-37.4)	9	94	6.4	0.84	0.48
34.8(32.6-36.0)	8	130	12	1.5	5.2
27.6	1	205	14	1.8	6.8

[1] correlation significant for HCB ($P < 0.005$), DDE ($P < 0.005$) and PCB ($P < 0.005$), not significant for dieldrin ($P > 0.05$). Kendall Rank correlation test according to Wilcoxon and Wilcox (1964).

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Many data from the literature, which will not be reviewed here, strongly suggest that DDE could be one of the main causal factors involved in egg shell thinning in wild birds.

The presence of PCB in cormorant tissues was confirmed by mass-spectrometry. Apart from PCB a new environmental contaminant was detected in the tissues of the cormorant. In a previous study (Koeman et al., 1969) we reported the presence of a compound with massnumber 376 and 8 chlorine atoms per molecule in the tissues of eider ducks. By using a computer GC/MS system and comparison with reference compounds the unknown substance could be identified as octachlorostyrene. Two heptachlorostyrenes were also found (ten Noever de Brauw and Koeman, 1973). Quantitative estimations are in progress. Polychlorinated dibenzofurans were not detected so far.

As can be seen in table 4 comparably high concentrations were also found in herons. An experimental study with three herons which were poisoned with PCB under experimental conditions revealed that this species apparently is less susceptible than the cormorant. A much higher dose rate had to be applied in order to induce symptoms. At death the following concentrations were recorded in brain and liver. Brain: 420, 430 and 445 ppm respectively; liver: 1140, 1275 and 1430 ppm respectively. The adipose tissue of the latter bird contained 56,400 ppm. As in the case of the cormorant the characteristic symptoms observed in various laboratory animals after PCB poisoning were not found in the heron.

4. <u>PCB in seals, porpoises and dolphins.</u>

During 1970 and 1971 a number of common seals (<u>Phoca vitulina</u>) and harbour porpoises (<u>Phocoena phocoena</u>) found dead along the Dutch coast were analysed for PCB and other compounds (in particular metals).

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In addition two bottle-nosed dolphins (Tursiops truncatus) were analysed which died in a dolphinarium in the Netherlands. By mediation of the Zoological Museum and Zoological Laboratory of the University of Amsterdam samples were obtained from common dolphins (Delphinus delphis) and dusky dolphins (Lagenorhynchus obscurus) caught in fishing nets along the coast of New Zealand and a Surinam dolphin (Sotalia guianensis) which also died in a fishing net.

The results of the blubber (subcutaneous fat) analyses of 7 seals are presented in fig. 1. It can be seen that the PCB levels do far exceed those of DDT. The difference between DDT and PCB levels is far less for the porpoises (fig. 2.). In absolute terms the concentration of the DDT group compounds (DDT + DDE + DDD) was higher in porpoises than in seals. In 1968 Risebrough reported that the DDT/PCB ratio appeared to be higher in marine birds from remote areas than in those caught in less remote areas (Risebrough et al., 1968).

Considering that seals generally live much closer to the shore than porpoises, the higher DDT/PCB ratio for the latter group is well in agreement with Risebrough's finding. The levels in the blubber of the bottle-nosed dolphins were of the same order as those in porpoises. It is interesting to note that PCB's could not be detected in the blubber of the dolphins from New Zealand and Dutch Guiana (fig. 3.). However, the DDT group levels in these Pacific dolphins are of the same order of magnitude as the levels found in porpoises from the North Sea.

The toxicological evaluation of the PCB in the tissues of seals and whales cannot be made at present. In the adult seals the brain levels varied from 13 to 89 ppm on a wet weight basis.

Recently Ringer et al.(1972) reported levels of 11 ppm in mink (Lutreola lutreola) which died after being fed either dietary PCB containing coho salmon or ocean fis with supplemental PCB (basal

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diet supplemented with 10 ppm each of Aroclors 1242, 1248 and 1254). The authors also found an effect on reproduction (serious drop in number of producing females and kit mortality)(see also Aulerich et al., 1971). As compared to these data the levels in the marine carnivorous mammals reported above merit serious attention. The data on metals were reported elsewhere (Koeman et al., 1972b).

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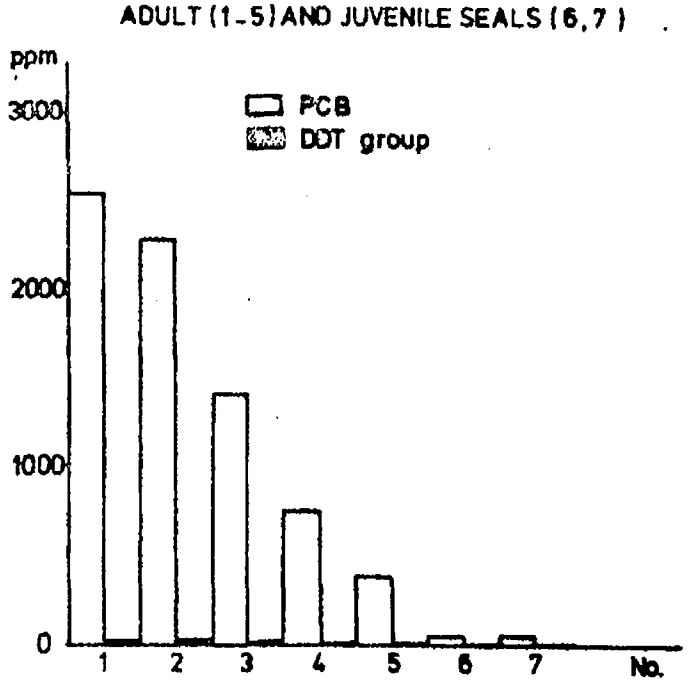

fig. 1. PCB and DDT group concentrations in blubber (subcutaneous fat) of 7 seals found dead in 1970 and 1971 (the hexane extractable lipid content varied from 19-68 %)

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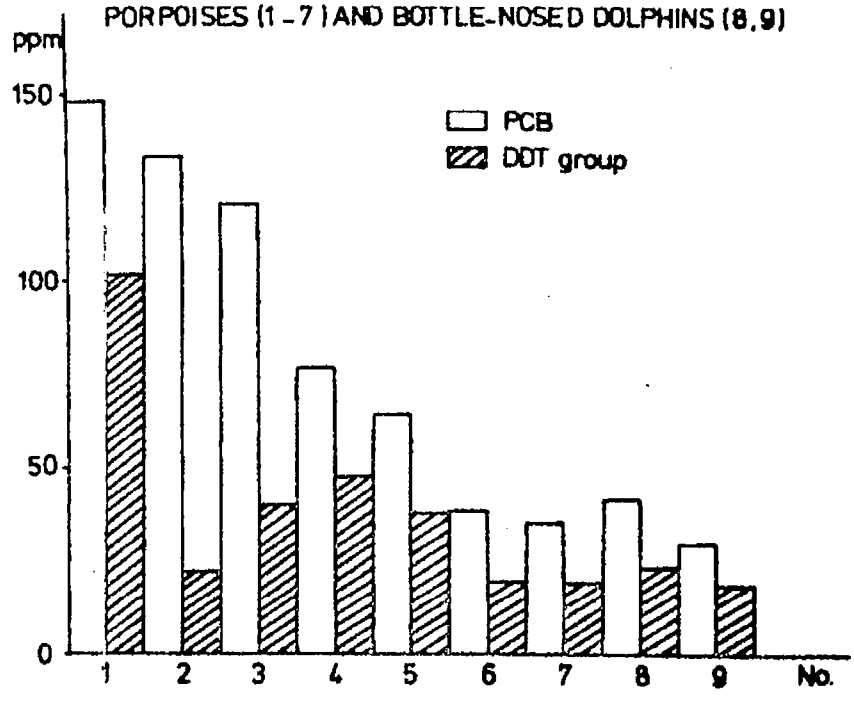

Fig. 2. PCB on DDT group concentrations in blubber of stranded porpoises and bottle-nosed dolphins from a dolphinarium (the hexane extractable lipid content varied from 64-96 %)

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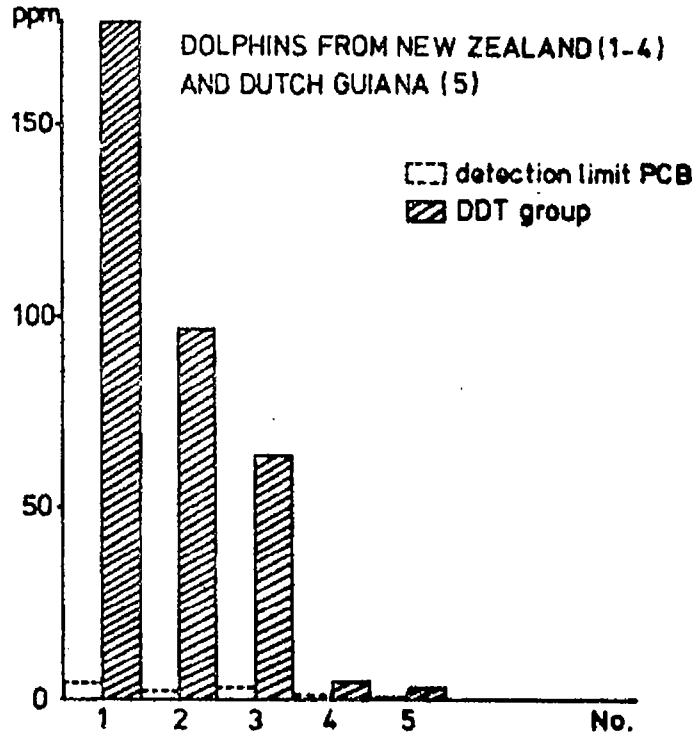

fig. 3. DDT group concentrations in blubber of dolphins caught in fishing nets in New Zealand and Dutch Guiana (the hexane extractable lipid content varied from 40-80 %).

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PCB Conference II
Stockholm 1972
General Discussion

Photochemical degradation of polyhalogenated biphenyls

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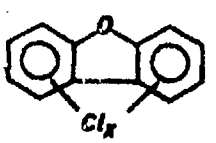
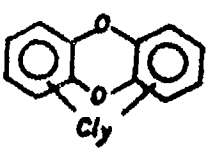
S-901 87 Umeå, Sweden

In 1970 it was reported by Vos and coworkers that technical PCB preparations contained toxic, polar components, identified by mass spectroscopy as polychlorinated dibenzofurans (1).[1]



x = 4, 5

1



y = 2–8

2

From a chemical point of view these chlorodibenzofurans are closely related to the polychlorinated dibenzodioxins (2) known as toxic and teratogenic contaminants in chlorophenols and chlorophenoxy acids.

PCB is known to be stable and resistent to chemical and microbial breakdown. Therefore photochemical degradation induced by the UV-content in the sunlight may be a major pathway of decomposition. Last year Safe and Hutzinger gave a report on the photochemical

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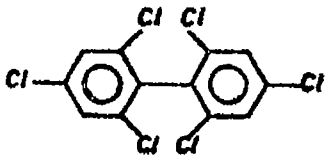
degradation of 2,2',4,4',6,6'-hexachlorobiphenyl (3). Among the products



3

they found one with the molecular weight of 304.[2] This is the calculated molecular weight for tetrachlorodibenzofuran (1, x=4).

In subsequent papers the same group has studied the photochemical decomposition of individual PCB's as well as technical PCB preparations. In all cases they obtained polar oxygenated products, but no real effort seems to have been made to separate and identify these products,[3,4] which must be an investigation of great importance.

From work with identifying impurities in pentachlorophenol we had some experience from the separation and identification of as well as reactions with polychlorinated oxygenated aromatics.[5]

We started to investigate the photochemical behaviour of the Cl_6-PCB 3. We used methanol as solvent and a lamp λ_{max} = 290 - 430 nm which is close to the solar radiation, and the sample was irradiated for 30 min.

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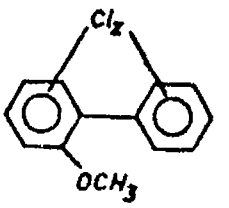
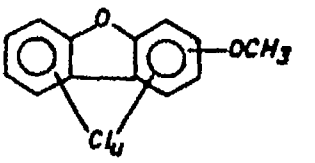
The first separation of the variety of products formed was made by thin layer chromatography, and we could detect at least nine bands. Each band was eluated and the eluate analyzed by means of a combination of gas chromatography - mass spectrometer.

As being reported before we found dechlorination to be one main decomposition pathway. We could identify PCB's with 5,4,3,2 and 1 chlorine. Among the oxygenated products we were able to identify a series of o-methoxy-PCB's (4) and



$z = 1-5$

4



$u = 1-4$

5

another group of compounds we consider to be methoxysubstituted chlorodibenzofurans (5).

We discovered that around 80 compounds were formed during this irradiation, and the majority of these were oxygenated chloroaromatics. We know very little about the toxic, genetic, cancerogenic and teratogenic effects of these compounds.
Flame retardants are in general halogenated compounds and some of them are very closely related to PCB. The consumption of flame retardant is expected to increase significatly. Therefore we feel that special interest must be devoted to these compounds.

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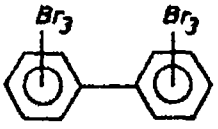
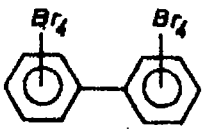
Hexabromobiphenyl (6) is known under the trademark fireMaster BP 6



6



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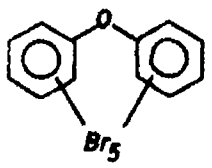
and the octabromo compound 7 as Bromkal 80. In addition to 6 and 7 two polybrominated diphenylethers 8 and 9 have been introduced as flame retardants for polymers. In this connection it must be worth



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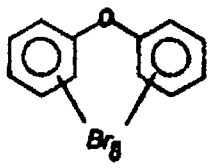

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notice that Suschitzky recently reported that the azadibenzofuran 11 can be prepared in preparative yield by a photochemical cyclisation of the chlorinated ether 10.[6]



10 11

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DSW 038646

STLCOPCB4022470

THE DISTRIBUTION AND METABOLISM OF LABELLED POLYCHLORINATED BIPHENYLS IN MICE AND QUAILS:

By

Bengt Melvås and Ingvar Brandt

DSW 038647

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An extensive investigation has been started with the aim of studying and comparing the distribution and metabolism of several different polychlorobiphenyls varying with respect to number and position of chlorine atoms.

With this goal in view the synthesis of a number of ^{14}C-labelled compounds has been planned and started.

The distribution of these substances is being studied by whole body autoradiography in mice and birds and the biotransformation is simultaneously looked into.

So far a mixture of 2,3,4,2^1,4^1,6^1- and 2,4,6,3^1,4^1,5^1-hexachlorobiphenyl (I + II) and a mixture of 2,4,2^1,3^1- and 2,4,3^1,4^1-tetrachlorobiphenyl (III + IV) have been labelled by Wachtmeister. Labelled 2,4,2^1,4^1-tetrachlorobiphenyl (V) has been synthetized by Melvås.

Preliminary results from the investigations of tetrachlorobiphenyls in mice (V) and in quails (III + IV) are obtained and a review of the biological data so far available will be given.

DSW 038648

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IDENTITY AND PURITY OF C^{14}(U)-LABELLED-2,4,2^1,4^1-TETRA-CHLOROBIPHENYL.

C^{14}(U)-labelled 1-chloro-2,4-dinitrobenzene with a spefific activity of 25 µCi/mg were coupled accor dng to Ullman (2) to 2,4,2^1,4^1-tetranitrobiphenyl. The nitro groups were then substituted with chlorine giving 2,4,2^1,4^1-tetrachlorobiphenyl (Figure 1 synthesis I). Melvås (3,4).

The purity was tested with gas-chromatography and thin layer chromatography. To confirm the structure we synthetized the same 2,4,2^1,4^1-tetrachlorobiphenyl with Ullman-coupling starting with unlabelled 1-iodo-2,4-dichlorobenzene (Figure 1 synthesis II). Mass spectra were taken both on the labelled and unlabelled 2,4,2^1,4^1-tetrachlorobiphenyl.

Gas chromatography.

The gas chromatographic separations were made on a Varian 204 gas chromatograph.

Column: 1/8" m stainless steel.

Support: Chromosorb W AW DMCS 80/100 mesh.

Liquid phase: 2% SE-30.

Carrier gas: Nitrogen.

Flow rate: 20 ml/min.

Detector: Flame ionisation.

Detector temperature: 270°C.

Injector temperature: 250°C.

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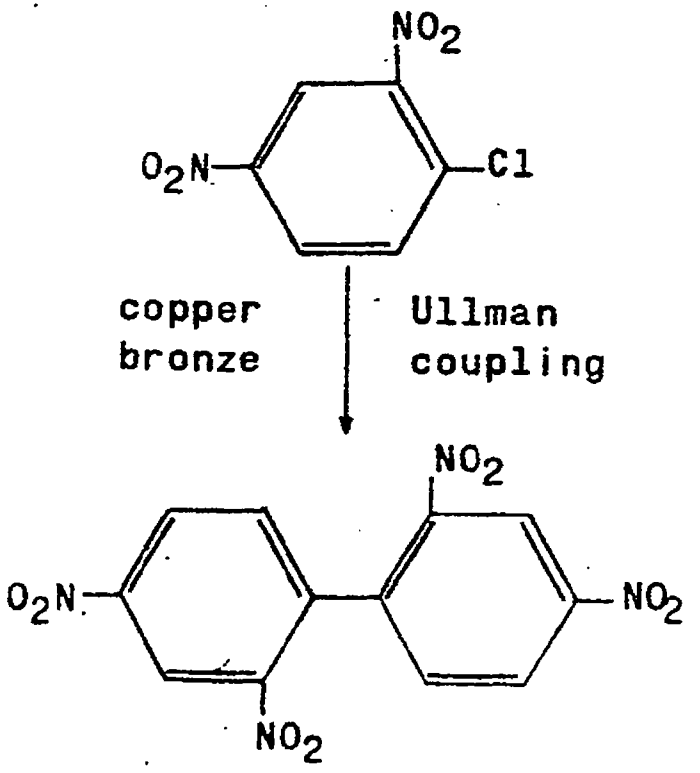
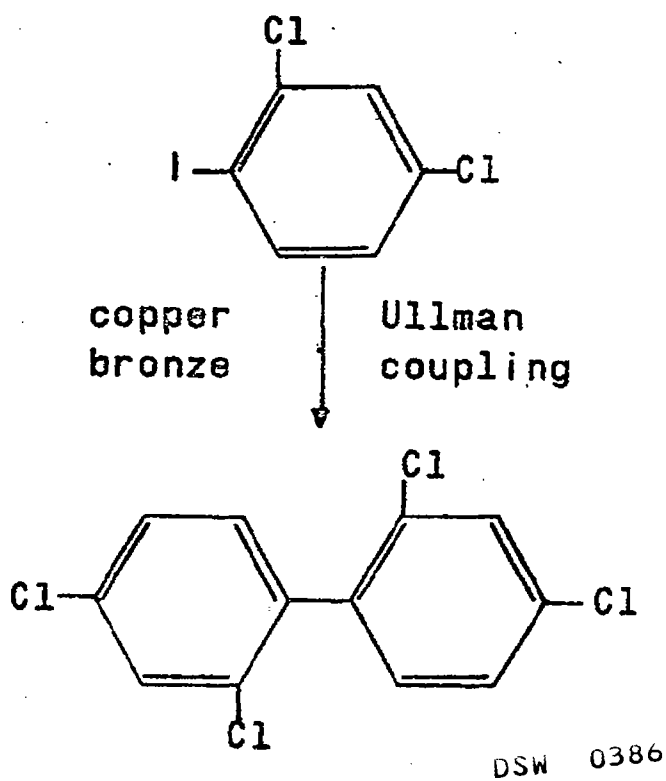
Synthesis I C^{14}(U)

1-chloro-2,4-dinitrobenzene



Synthesis II (unlabelled)

1-iodo-2,4-dichlorobenzene



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Flame ionisation detector was preferred instead of electron capture detector in order to detect impurities others than those with high electron affinity. Both isothermal and programmed modes were used. When programmed from 80°C to 260°C only one single peak could be seen on the chromatogram. The tetrachlorobiphenyl was eluted at 179°C.

Mass spectrometry.

That lot of the labelled 2,4,2',4'-tetrachlorobiphenyl injected in the mice series were analysed by mass spectrometry and so was the unlabelled 2,4,2',4'-tetrachlorobiphenyl synthetized from dichloro-iodo-benzene. Both mass spectra are identical within the error of the instrument.

Mintspectra of 2,4,2',4'-tetrachlorobiphenyl.

M/e	Starting materials 1-chloro-2,6-dinitro-benzene ^{37}Cl (U) (relative peak height in %)	1-iodo-2,4-dichloro-benzene (unlabelled) (relative peak height in %)
110	17.2	13.5
150	22.4	20,2
151	14.0	13.1
220	68.6	67.4
222	64.7	48.3
290	76.0	79.5
291	15.7	16.7
292	100.0	100.0
293	16.2	16.7
294	46.6	49.0

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Mass spectra of
2,4,2',4'-tetrachlorobiphenyl (C^{14} U)
starting from 2,4-dinitrochlorobenzene (C^{14} U)

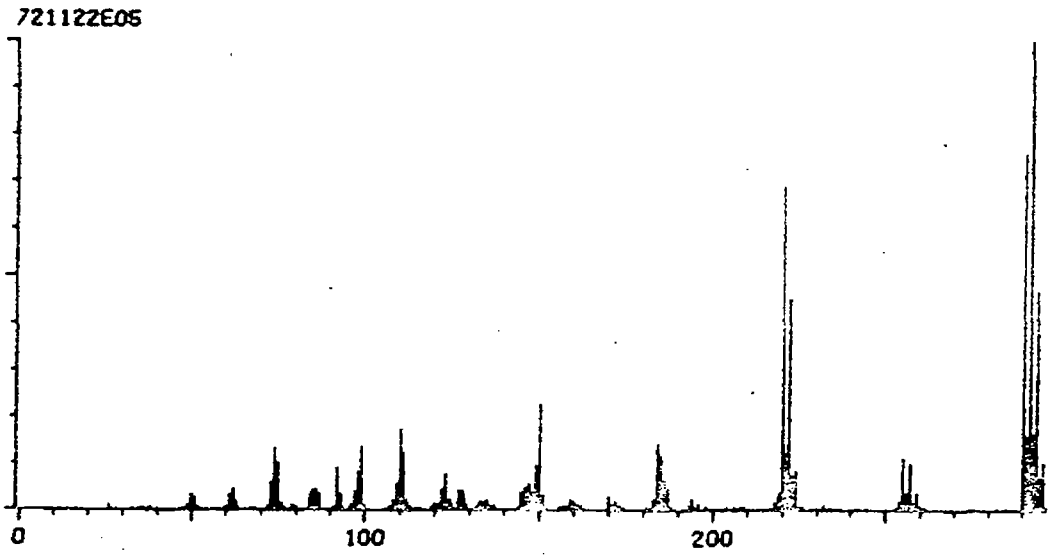

Figure 2

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Mass spectra of
2,4,2',4'-tetrachlorobiphenyl (unlabelled)
starting from 1-iodo-2,4-dichlorobenzene

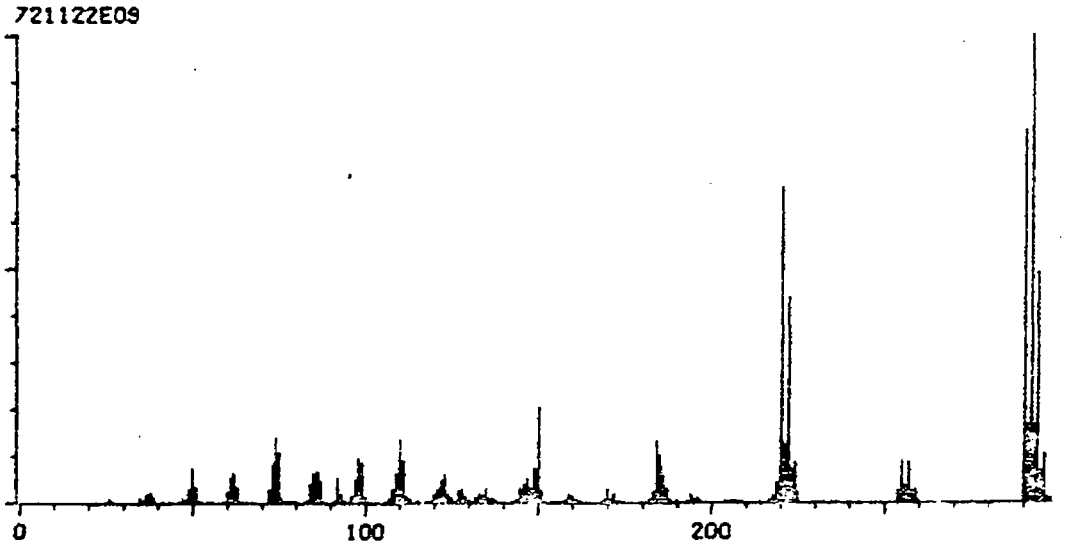

Figure 3

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Mass spectrometric conditions:

Ionisation voltage: 70 e V

Ionisation source temperature: 285°C

Separator temperature: 250°C

Column-liquid fase: OV 1

He flow: 20 ml/min.

The mass spectra were done on an LKB-GC-mass spectrometer at the crystallographic group, Institute of medical chemistry, University of Gothenburg.

Thin layer chromatography

Pre-coated 0.25 mm silicagel TLC-plates with fluorescent indicator F-254, 10 x 20 cm. (Merck 5729/0050) were used.

With heptane as a moving phase the radioactive compound got a RF-value of 46.

Autoradiography of the plate showed only one spot. No fluorescent spot could be detected at inspection in UV-light.

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Mass spectrometric conditions:

Ionisation voltage: 70 e V

Ionisation source temperature: 285°C

Separator temperature: 250°C

Column-liquid fase: OV 1

He flow: 20 ml/min.

The mass spectra were done on an LKB-GC-mass spectrometer at the crystallographic group, Institute of medical chemistry, University of Gothenburg.

Thin layer chromatography

Pre-coated 0.25 mm silicagel TLC-plates with fluorescent indicator F-254, 10 x 20 cm. (Merck 5729/0050) were used.

With heptane as a moving phase the radioactive compound got a RF-value of 46.

Autoradiography of the plate showed only one spot. No fluorescent spot could be detected at inspection in UV-light.

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ANIMAL EXPERIMENT 8

Metabolism.

The mice used in this series were of C57 black strain. Each mouse received in a tail vein an injection of 6 µCi 2,4,2^1,4^1-tetrachlorobiphenyl dissolved in 30 µl DMSO. The specific activity was 28 µCi/mg. Faeces and urine were collected in metabolism cages during 24 hour periods and stored in closed vials. In order to get a proximate figure of the relative rate of faecal excretion aliquots of each 24 hour faeces collection were placed under a GM-tube and counted under the same geometrical conditions.

The samples were measured three times, each time 200 or 800 secs and the average plotted (Figure 4). It can be seen that the amount excreted with the faeces from mice reaches a maximum a few days after the administration. This is in accordance with the autoradiographic data.

Of the collected faeces 500 mg samples were taken and extracted with diehylether (Merck zur Analyse) in a Soxhlet extractor with 8 ml sample capacity. The ether extract was evaporated to 2 ml. The concentrated ether solution was analyzed with thin layer chromatography.

Following elution media were used: chloroform, chloroform-methanol (1:1), methanol, heptane-aceton (2:1), heptane and pentane.

Tetrachlorobiphenyl has an RF-value on silica gel with heptane as moving fase of 46, but the metabolites do not move at all. Comparative studies indicate a very close resemblance with hydroxybiphenyls.

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The metabolites were indicated with autoradiography of the thin layer plates. The active compounds were scraped off and extracted for further analyses with gas chromatography and mass spectrometry.

2,4,2^1,4^1-tetrachlorobiphenyl and the mixture of 2,3,2^1,4^1- and 3,4,2^1,4^1-tetrachlorobiphenyl give both the same general excretion rates with a maximum at the first day and they also give metabolites with the same RF-value if chromatographed on silica gel with heptane: acetone (1:1). The mixture of hexachlorobiphenyl has not yet been analyzed for metabolites but the faeces activity counting indicates a longer retention time than was the case for the tetrachlorobiphenyls.

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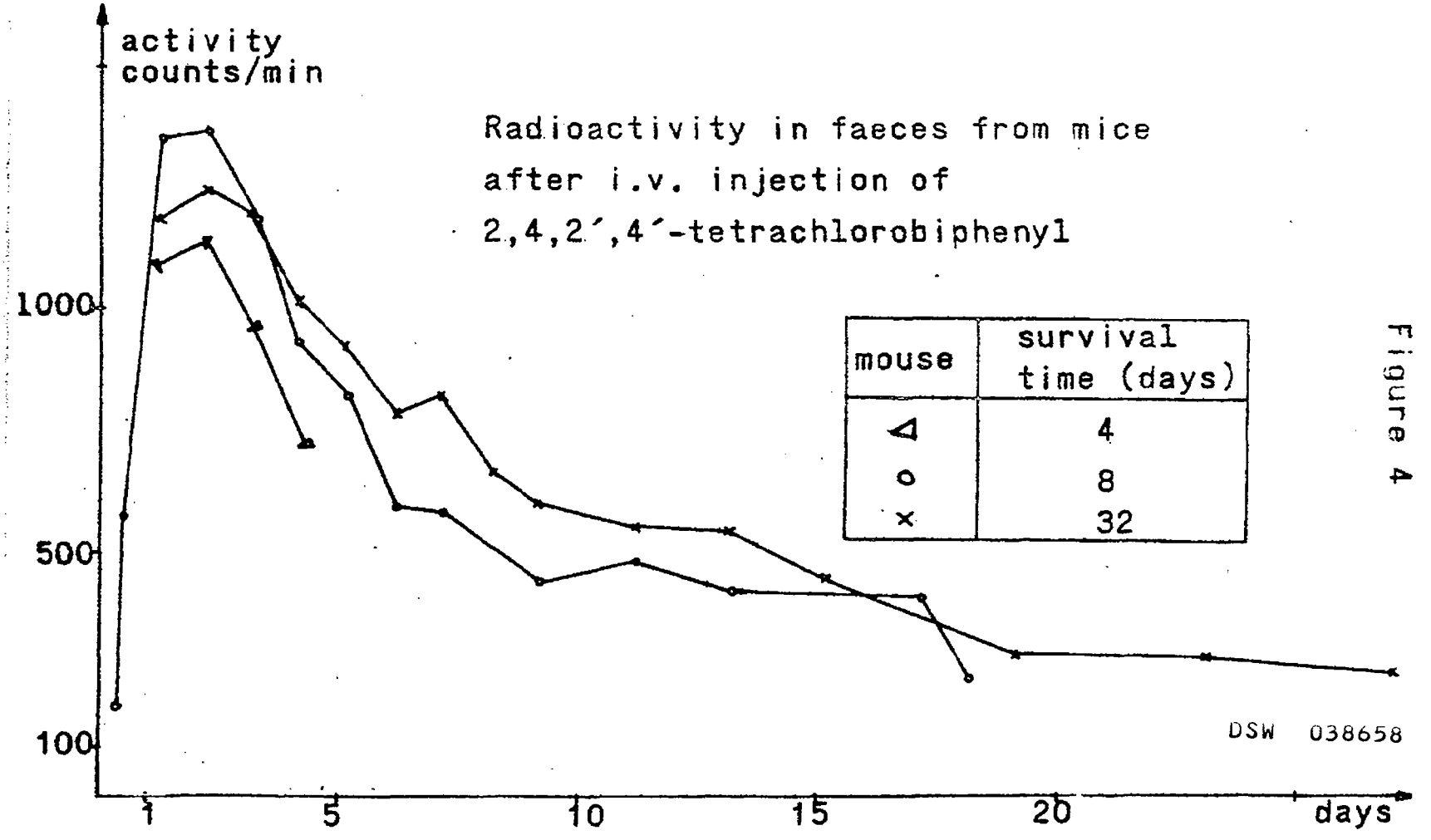

Figure 4

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AUTORADIOGRAPHY (preliminary results)

A series of mice, strain C57 black, were injected with 6 µCi 2,4,2^1,4^1-tetrachlorobiphenyl each in a tail vein. The substance corresponding to 0,12 mg was dissolved in 30 µl dimethylsulfoxide. The animals were killed after survival times from four minutes to 32 days. At the time of sacrifice the animals were anesthetized in chloroform and mounted in a gel of carboxymethyl cellulose and water. Then they were frozen to solid blocks in hexane cooled with solid carbon dioxide.

A series of male Chinese dwarf quails, Excalfactoria chinensis, weighing about 40 g each were injected with a preparation containing a mixture of 2,4,2^1,3^1- and 2,4,3^1,4^1-tetrachlorobiphenyl in a radial vein. The dose for each animal was 12 µCi corresponding to 0,14 mg. After survival times varying from four minutes to 30 days the birds were killed by anesthesia in carbon dioxide and plucked. Then they were frozen and mounted for sectioning as the mice.

Another series of laying Japanese quails, Coturnix coturnix japonica, weighing about 140 g each, were injected with 20 µCi of the same preparation and then anesthetized and frozen. Saggital sections attached to tape were taken at different levels of the body. The sections were dried at -15°C for a few days and then attached to X-ray film. Following exposure time up to eight months the autoradiograms were developed.

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Distribution in mice (2,4,2^{1},4^{1}-tetrachlorobiphenyl) and in quails (2,4,2^{1},3^{1} and 2,4,3^{1},4^{1}-tetrachlorobiphenyl).

Main findings in both species:

The most striking observation in the distribution pattern found in the mice was a very pronounced accumulation in the adrenal cortex and the corpora lutea of the ovaries. Short time after injection the concentration in these areas was considerably higher than in the fat but at later times the fat depots showed higher concentration than any other tissues.

High activity was seen in the salivary glands, Harder's gland and in the gastric mucosa in mice short time after injection.

The concentration was also rather high in the liver throughout the investigation period.

The concentration in the central nervous system was considerably lower and the level in the fetuses never approached the maternal.

In the quails the highest concentration in the body was found in the yolk 5 days after injection. As in the mice the fat and liver also were dominating.

In both the mice and the quails there was a rapid biliary excretion of radioactive material to the small intestine, where high activity was seen long time after injection.

Mouse (2,4,2^{1},4^{1}-tetrachlorobiphenyl)

4 min: Following intravenous administration the substance rapidly left the bloodstream. Four minutes after injection the activity in most tissues exceeded the blood level. The most dominating

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accumulation at this survival time occurred in the adrenal cortex, and in the corpora lutea in the ovaries.

In the liver gastric mucosa and in the kidneys there was rather high activity.

Also in the salivary glands and in Harder's gland a pronounced accumulation was observed.

The concentration in the central nervous system was slightly higher than in the blood, but considerably lower than the myocardium. At this survival time the accumulation in body fat was below the liver level.

40 min: Forty minutes after administration the uptake in fat depots especially in the brown fat had increased compared to the liver. The activity in the gall bladder now exceeded that of the liver.

In the kidneys the concentration had decreased and now was of about the same magnitude as in the central nervous system. In the mammary glands the activity was slightly above the kidney level.

Labelled substance passed the placenta but the fetal concentration was considerably lower than the maternal. Within the fetuses the highest levels were found in the livers.

4 hrs: Four hours after injection the activity in the liver was beginning to decrease. The autoradiograms tended to be more dominated by the fat depots. In the liver the gall bladder contents showed activity far above that of the liver. Very heavy activity was seen in the intestinal contents apparently due to

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biliary excretion. In the [illegible] nervous system the concentration had increased compared to the liver. The distribution pattern was becoming more distinct, especially in the cerebellum where a tendency to higher activity in the white matter could be seen.

24 hrs: Twentyfour hours after injection the fat depots were still more dominating. In the liver tissue a marked reduction in activity had occurred, while the concentration in the bile was at least of the same magnitude as in the subcutaneous fat.

4 and 8 days: Four and eight days after administration the level labelled substance retained in the fat depots was high compared to the liver. The activity in the central nervous system was very low at these survival times.

Quails.

The distribution in the cock quail following injection of 2,4,2^1,3^1 and 2,4,3^1,4^1- tetrachlorobiphenyl was as a whole in agreement with that of 2,4,2^1,4^1-tetrachlorobiphenyl in mice.

1 h: The activity in the blood did not decrease as fast as in the mice. One hour after injection the level active substance in the heart blood was about the same as in the myocardium and pectoral muscle.

The activity in the central nervous system was exceeding that of the blood. In the cerebellum the uptake gradually tended to be concentrated to the white matter.

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A fairly high concentration of labelled substance was seen in the liver. In the kidneys the activity was about the same as in the central nervous system.

In the body fat the accumulation was high at this survival time. The most dominating concentration was, however, seen in the intestinal contents.

Also in the gizzard contents heavy concentration of labelled material could be seen, while the gizzard wall itself was almost lacking activity.

In the testes, the concentration was considerably lower than in the heart blood, but larger than in the gizzard wall.

Evidence for specific localization to endocrine organs has not been found.

4 hrs: Four hours after administration the fat accumulation was becoming more dominating in the autoradiograms together with the high level active substance excreted to the gizzard and intestines.

Autoradiograms from longer survival time were even more dominated by the retention in fat depots and excretion to the intestinal channel. Four days after administration the activity in the bile was far above that in the liver tissues.

Hen.

In the laying hen the distribution pattern was about the same as in the cock. The blood levels were, however, at all survival times higher, compared to the myocardium.

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Labelled substance was heavily taken up and retained in the yolk of the follicle and egg. Five days after administration the activity in yolk exceeded all other tissues in the body. Active substance was poorly taken up by the albumen of the egg.

One hour after injection the activity in the oviduct was almost the same as in the kidney, but not so high as in the blood.

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DISCUSSION

Polychlorinated biphenyls are established to be potent inducers of microsomal liver enzymes, increasing the metabolic rates of different steroid hormones (Risebrough et al. Lincer et al.). Different physiological disturbances following exposure to low doses polychlorinated biphenyls have been related to this phenomenon.

The most remarkable finding in this investigation was a very pronounced accumulation of labelled material in the adrenal cortex and corpora lutea in mice four minutes after injection of $2,4,2^{1},4^{1}$-tetrachlorobiphenyl. At this survival time the radioactivity in these sites exceeded all other tissues in the body. This rapid and selective uptake suggests a possible direct interference in the metabolism of steroid hormone secreting cells.

Following a daily dose of 70 mg technical PCB (kanechlor 400) per kg body weight for a month to rats, atrophy in the adrenal cortex was demonstrated (Inao et al.). The corticosterone producing capacity of the adrenals was significantly decreased. In cockerels fed 250 ppm Arochlor 1254 in the diet an antiandrogen effect, assessed by a decrease in testicular and comb growth, was observed (Platonow et al.).

Following injection of PCB (20 mg/kg) in mice prolonged estrous cycles were determined (Örberg et al.).

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By means of the 18 hrs glycogen response in the immature rat uterus an estrogen effect was demonstrated following injection of different Arochlors containing up to 48% chlorine (Bitman et al.).

It is thus well established that low doses polychlorinated biphenyls reveals hormonal effects. It seems resonable to assume that the disturbances in the steroid metabolism might be accomplished in two different ways. An increased hydroxylating capacity due to stimulation of microsomal enzymes in the liver gives more polar metabolites to be excreted. Besides, a direct toxic effect on the steroid hormone synthetizing organs, leading to an altered and/or decreased hormone production, is possible. The toxicology of polychlorinated biphenyls exhibit many similarities to that of DDT and its metabolites. DDT was shown to accumulate in the adrenal cortex and ovaries in mice (Bäckström et al.). Adrenal cortical atrophy and even necrosis was demonstrated in dogs following ingestion of DDD (Nelson et al.).

The corticosterone production in chicks was found to be inhibited by feeding subtoxic amounts of technical grade DDT. The degree of reduction was as a rule proportional to the dose given or to the concentration of DDT metabolites found in the adrenals (Srebocan et al.).

In the present investigation no uptake of labelled tetrachlorobiphenyl in endocrine organs has so far been observed in birds.

The distribution pattern of tetrachlorobiphenyl is characterized by its fat solubility. Within the first hour after injection the concentration in fat depots was very high. In spite of decreasing

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activity in the fat tissues the autoradiograms from the longer survival times were dominated by these areas.

In the intestinal contents labelled substance was found already four minutes after injection in mice. Forty minutes after injection the radioactivity in the gall bladder was higher than in the liver tissue, indicating a rapid biliary excretion to the small intestine. Since at least the nonmetabolized polychlorinated biphenyls are almost completely absorbed from the intestines following peroral administration (Albro et al. it seems likely that a considerable enterohepatic circulation occurs.

In the laying hen labelled substance was heavily accumulated and retained in the yolk of the follicle and egg afer a single injection. It is obvious that small amounts of PCB this way might be transferred to the embryo and thus possiby cause disturbances in the development.

After administration of 50 mg Arochlor 1254 weekly to pheasants no significant difference in the hatchability, compared to a control group, could be seen. The number of eggs that were pipped but not hatched, however, increased significantly indicating a decreased viability of the embryo at the time of hatching (Dahlgren et al.).

Following injection of 10 mg Arochlor 1242 into embryonated White Leghorn eggs, only five percent of the eggs were hatched. Investigation of the dead fetuses revealed beak deformities, edema and growth retardation (McLaughlin et al.).

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PCB Conference II
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The Metabolism and Distribution of 2,4,5,2',5'-Pentachlorobiphenyl in the Mouse.

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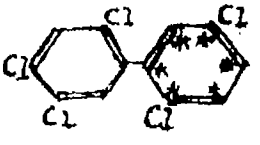
2,4,5,2',5'-Pentachlorobiphenyl is a major constituent of Arochlor 1254, and it forms a minor component of the PCB content of human fat (Jensen, personal communication). A sample labelled with ^{14}C (30 mCi/g) was obtained from Mallinckrodt; it was supplied in benzene solution (0.67 mCi/ml) and gave a single PCB peak by gas-liquid chromatography.



2,4,5,2',5'-Pentachlorobiphenyl
(* ^{14}C-labelled)

This communication is an interim report of an investigation which includes a whole-body autoradiographic study of the distribution of radioactivity in mouse tissues, and the measurement of radioactivity in tissues and excreta after a single oral or intravenous dose of the ^{14}C-PCB.

METHODS

Preparation of the dose

The PCB was administered to CBA mice as a solution in dimethylsulphoxide (DMSO) or in an aqueous emulsion. For the DMSO so-

x) Names are intentionally presented in alphabetical order

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lution, a measured volume of the PCB solution was evaporated to dryness and the residue dissolved in sufficient DMSO to give 10 µCi in 0.05 ml. For the emulsion, a measured volume of the benzene solution was evaporated in a polypropylene tube and the residue dissolved in the lipid-phosphatide phase of "Intralipid" 20% (Vitrum). This solution was emulsified in 4 volumes of 2.5% aqueous glycerol by pumping it several times through a needle attached to a syringe, and this primary emulsion was then homogenized by placing the tube in a Varian ultrasonic bath maintained at 60°. The droplet size of the emulsion so produced was about the same as that of "Intralipid", most droplets being less than 1 µm diameter.

Whole-body autoradiography

For the whole-body autoradiograms, a dose of 10 µCi of the DMSO solution or of the emulsion was injected into the tail vein, and 10 µCi of the emulsion was administered orally. This dose corresponds to about 15 mg/kg body weight of PCB. Animals were killed 20 min., 1 hr, 4 hr, 1 day and 35 days after the dose, and whole-body autoradiograms were prepared by the method of Ullberg (Ullberg, S., Studies on the Distribution and Fate of S^{35}-Labelled Benzylpenicillin in the Body, Acta Radiol. (Stockh.) Suppl. 118, 1954).

Determination of radioactivity in tissues and excreta

Mice were dosed orally with 5 µCi PCB emulsion and groups of three were killed 1, 4, 8 and 16 days after dosing, and the organs removed for radioactivity measurements. Organs were also included from the mice dosed with 10 µCi for the autoradiographic experiments. Another group of three mice each receiving 5 µCi was maintained in a metabolism cage and the urine and faeces collected separately.

A sample of each tissue was weighed on filter paper, dried and ignited in an oxygen flask containing 1 M sodium hydroxide solu-

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tion. The daily output of faeces was dried and weighed, then ground in a mortar and a weighed amount similarly ignited in the oxygen flask. Diluted urine and the sodium hydroxide solutions were added to Instagel and the radioactivity measured with a Packard Series 3000 Scintillation Counter.

RESULTS

Whole-body autoradiography

20 min. After the intravenous DMSO injection, most of the radioactivity had entered the tissues from the blood within 20 min. The activities of fat and of the liver were the highest and about equal. After the emulsion injection the passage into the tissues was slower but the concentration in fat and in the liver was greater than in blood. With both methods of administration the radioactivity in the kidney was localized in specific areas. Radioactivity was clearly present in the stomach contents.

1 hr. After 1 hr there was no difference between the distribution of radioactivity obtained with the DMSO and emulsion intravenous injections. The highest activity was seen in brown fat, with rather less in liver. Radioactivity is clearly present in the contents of the small intestine and of the transverse colon; there was some in the white pulp of the spleen but none in the pancreas.

4 hr. Little radioactivity was left in blood. Most was seen in fat and in the intestinal contents, with the liver as the next most active tissue.

1 day. Fatty tissue was clearly the most radioactive, followed by the contents of the intestines. Some sections including the gall bladder and the bile was seen to contain radioactivity. Other sections showed spots on the liver which may indicate the bile ducts. There was a peculiar pattern in the lung with a concentration in some structure not yet identified. The activity in brain

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had decreased. There were radioactive areas in the white pulp of the spleen, in the parotid and lachrymal glands and in the epithelium of the nasal sinuses. There was some radioactivity in the foetal membranes, with a very slight penetration into foetal tissue.

Sections from a mouse killed 1 day after an oral dose of emulsion showed a very similar distribution of radioactivity. There was a similar pattern in the lungs, which may be interpreted as an accumulation in the bronchial and bronchiolar epithelia.

35 days. Most radioactivity had left the animal after this period. The most clearly defined areas were the lungs and the renal cortex; the concentration in fat appeared to be lower, with rather less in brown fat than in white fat.

Radioactivity measurements in tissues and excreta

The radioactivity in the pooled urine and faeces of 3 mice each dosed with 5 µCi, expressed as a percentage of the dose, is shown in Table 1. From these results it can be calculated that the half-time of the PCB in the body is about 7 days.

Table 2 shows the radioactivity in the organs at various periods after dosing. The results obtained from mice used in the autoradiographic experiments are not strictly comparable, as the dose was twice as large. At present, the results on only one animal from each group are available. One pregnant female dosed intravenously with 6 µCi showed 0,7 nCi/100 mg in the foetus after 1 day..

DISCUSSION

The results show that the uptake of PCB by the tissues from the circulation is rapid and not much delayed if the PCB is administered intravenously in the lipid phase of an aqueous emulsion. This

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method of administration is free from some of the disadvantages associated with the use of organic liquids as solvents for intravenous injections.

Throughout the experiment, measurements of radioactivity show the highest concentration to be in fat. The radioactivity in fat rises to a maximum between 4 and 24 hr after dosing, at a time when the radioactivity in blood is low and the concentration in tissues is decreasing rapidly. It must be assumed, therefore, that the PCB that is initially deposited in the tissues subsequently migrates to the fat and is stored there. Fat is confirmed as the major reservoir by the autoradiograms, although at 35 days the lungs and renal cortex appear to be more clearly defined, probably because the radioactivity is located in discrete areas. Radioactivity is lost fairly rapidly from the body with a half-time of about 7 days, and appears almost entirely in faeces. Autoradiograms show radioactivity in the contents of the intestines and gall bladder, so it seems likely that the major route of excretion is through the bile.

No information is yet available on whether the measured radioactivity is due to PCB or to a metabolite thereof; experiments are in progress to investigate this. It seems likely that the distribution seen at 20 min. after injection is due to unchanged PCB, and PCB may be stored as such in fat. However, it seems unlikely that PCB will be excreted in bile without undergoing some metabolic transformation, and the distribution in tissues other than fat at longer periods after injection may be due mainly to the metabolites. This may account for the sustained high concentration in liver and for the appearance of radioactivity in glandular secreting tissue and in certain areas of the lungs. The presence of radioactivity in the stomach contents may be due to excretion through the stomach wall or salivary glands, or to reflux of the duodenal contents.

No evidence of localization of radioactivity in specific areas of the brain can be observed in the autoradiograms. Analysis of

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brain tissue shows a low level of radioactivity which falls off during the experimental period, but a trace still remains after 35 days.

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Table 1. Excretion of radioactivity in urine and faeces

The results were obtained from pooled excreta of three mice, and are expressed as percentages of the dose administered (5 μCi/mouse).

Days after dosing	Faeces	Urine	Total	Cumulative total
1	20.3	0.23	20.53	20.5
2	9.9	0.11	10.01	30.5
3	4.1	0.12	4.22	34.8
4	7.0	0.13	7.13	41.9
5	3.6	0.17	3.77	45.7
6-7	5.4	0.16	5.56	51.2
8	3.6	0.13	3.73	55.0
9-10	6.4	0.11	6.51	61.5
11	2.3	0.07	2.37	63.8
12	1.8	0.10	1.90	65.7
13	2.5	0.08	2.58	68.3
14	2.2	0.09	2.29	70.6
15	2.0	0.09	2.09	72.7
16	2.3	0.06	2.36	75.1
17-18	2.4	0.12	2.52	77.6
19	1.6	0.06	1.66	79.2
Total	77.4	1.8	79.2	

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Table 2. Distribution of radioactivity in tissues after a single dose of ^{14}C-PCB.

Figures in Table represent μCi/100 mg tissue.

Dose, μCi/mouse	10[x]	10[x]	10[x]	5	5		5	5	10[x]
Route	iv	iv	iv	oral	oral		oral	oral	oral
Time after dosing	20 min	1 hr	4 hr	1 day	4 days		8 days	16 days	35 days
Sex	M	F	M	M	M	F	M	M	F
Blood	7.90	3.50	3.60				0.43	0.18	
red cells				0.40	0.16				
plasma				1.30	2.76	0.45			
Heart	12.50	9.90	13.00	2.18	0.50	0.37	0.41	0.57	
Lungs	27.00	14.80	5.70		3.10	3.15	1.90	0.22	2.28
Liver	50.90	33.40	12.20	6.50	2.80	1.80	3.60	0.78	0.64
Kidneys	19.30	9.80	8.40	8.60	1.70	1.50	1.00	0.50	0.76
Fat	4.30	13.30	11.90	27.90	10.70	14.00	13.40	8.20	3.30
Muscle	7.80	5.60	1.73		1.95	0.94	1.51	2.65	0.29
Brain	5.03	6.52	3.80	0.97	0.18	0.23	0.21	0.11	0.08

[x] Results have been halved to bring in line with 5 μCi experiments

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PCB - effects on mammalian reproduction

By

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Like many other chlorinated hydrocarbons PCB enhances the hepatic metabolism of sex steroids such as testosterone and estradiol (Peakall 1967, Nowicki and Norman 1972). It might therefore be suspected that the occurrence of PCB in animals would disturb the sexual functions and, perhaps, cause a decrease of the procreative ability of the animals, resulting in a continued decrease of the number of individuals per generation. From this point of view we have studied some sexual functions in mice of the NMRI-strain, given PCB in amounts corresponding to those found in wild life (Jensen et al. 1969, Jensen and Odsjö 1972).

1. Length of the estrous cycle.

The occurrence of heat in female animals is dependent upon the amount of estrogens circulating in the body. Consequently, a change in the catabolism of these hormones could be suspected to change the regular reappearing of heat periods. Therefore, we have studied the estrous cycle in mice by means of daily vaginal smears before and after one single intraperiotoneal injection of PCB (Clophen A 60, 20 mg/kg body weight). As seen from the following table the mean length of the estrous cycle increases upon administration of PCB (Örberg et al. 1972).

Length of the estrous cycle before and after one single injection of PCB (20 mg/kg body weight)

	Number of animals	Total number of cycles studied	Mean length in days Before injection	Mean length in days After injection	Analysis s	Analysis p
Control animals	24	96	5.6	5.6	1.81	0.04
Experimental animals	12	48	5.5	6.8		

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However, in the natural state PCB mainly enters the animals in food and water. For this reason we have given mice PCB orally, dissolving the drug in peanut oil. Mice happen to have a fancy for peanut oil and, consequently they consume the oil portions immediately and completely. The dose was 0.025 mg PCB/day/ /animal, approximately corresponding to 5 ppm in the food, and the experiments lasted for 62 days. The controls were given the corresponding volume of the peanut oil vehicle.

The results are given in the following table (Örberg and Kihlström 1972).

Effects of ingested PCB (0.025 mg/day/animal for 62 successive days) upon the length of the estrous cycle

	Number of animals	Total number of cycles studied	Mean length, days	Analysis	
				t	p <
Control animals	14	120	6.6	3.79	0.0005
Experimental animals	11	69	8.7		

The observed prolongation of the estrous cycle implies a prolonged interval between the short periods of sexual receptivity in females, probably resulting in a decreased number of pregnancies per individual.

2. Frequency of implanted ova

2.1. Females ingesting PCB as adults

The process of implantation of fertilized ova in the uterus demands a very subtle balance between progesterone and estradiol. This balance might be disturbed by an increased metabolism of these steroids. For this reason we counted the number of corpora lutea and implanted embryos in females ingesting 0.025 mg PCB/day/animal during 62 days before being mated and also during early pregnancy until being sacrificed on day 8-10 of pregnancy. The results given below (Örberg et al. 1972) show a decreased frequency of implanted ova in the treated animals.

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Frequency of implanted ova in females ingesting PCB (0,025 mg/day/animal)

	Number of animals	Number of corpora lutea	Number of ova implanted	Per cent of ova implanted	χ^2	p <
Control animals	22	288	250	87	5.60	0,025
Experimental animals	23	313	249	80		

2,2 Males and females sucking PCB-containing milk

It is well known (Harris 1965 and others) that the sex steroids present during the early postnatal period determine the development of the sexual functions and thus influences the reproductive ability of the adult animal. Sucking of PCB-containing milk during this period, possibly resulting in an increased catabolism of steroids, could therefore be deleterious to the sexual activity in the adults. In order to tackle this question we reared 20 different litters of mice. On the day of parturition ten mothers were given 50 mg PCB/kg body weight intraperitoneally and the ten remaining mothers the corresponding volume of the oil vehicle. The injections were repeated once a week throughout the period of lactation. When reaching sexual maturity the young animals coming from these litters were mated according to the scheme in the table below. The pregnant females were sacrificed on day 10-12 of pregnancy and the number of corpora lutea and placentae counted. The experiments are still going on, Consequently, the results in the following table are preliminary. When mating a control animal with an experimental one no effects upon the frequency of implantation have been observed so far. However, when both the animals of the couple had been sucking PCB-containing milk there is a significant decrease in the frequency of implanted ova.

Frequency of implanted ova in animals having sucked PCB-containing milk

Paired animals Male	Female	Number of pairs	Number of corpora lutea	Implanted ova Number	Per cent	Analysis χ^2	p <
Control	Control	16	211	199	94	-	-
Experimental	Control	17	216	200	93	0,27	0,7
Control	Experimental	19	240	219	91	1,13	0,3
Experimental	Experimental	21	262	191	73	35,57	0,0005

Thus, even small doses of PCB, given for short periods, may cause a decrease in the number of youngs per litter in mice.

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PCB Conference II
Stockholm 1972
General discussion

THE CONCENTRATION OF PCB IN HUMAN BLOOD AND ADIPOSE TISSUE IN THREE DIFFERENT RESEARCH GROUPS

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DSW 038681

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12 DEC 1972

The concentration of PCB in human blood and adipose tissue in three different research groups

S. Karppanen, L. Kolho

Today it has been definitely proved that PCB can be found everywhere in our surroundings and in living organisms. We also are learning constantly about its biological effects on different species. Knowledge about the harmful effects on human beings caused by PCB has been obtained chiefly from old sources or from accidents and we have very vague ideas about the doses or tissue concentrations that have any noticeable effect. Much confusion has also arisen because the commercial PCB-products have differences that concern PCB itself and especially impurities in them. In industrial toxicology it is often useful to know the tissue concentration of cumulative compounds and use it as an index of exposure which otherwise may be very difficult to estimate. Therefore we have determined the concentration of PCB in blood and in some cases also in subcutaneous adipose tissue of 29 persons.

Nine persons so far as we know have never been professionally exposed to PCB (group I, persons 1 - 9). Six other persons handle PCB samples in an analytical laboratory (group II, persons 10 - 15) and eleven persons have been working since 1968 at a capacitor factory where Arochlor 1242 is used for impregnation of capacitors (group III, persons 16 - 27). The PCB concentration in the air of this factory has been proved to fulfil internationally accepted limits. Special attention has been given to the protection of the skin.

The PCB concentration in blood and adipose tissue has been determined by gas chromatographic method which is presented in detail in the appendix of Matti Helminen's lecture. In figures 1 and 2 there are the results of the gas chromatograms of the blood and subcutaneous adipose tissue of some

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persons. The observed PCB concentrations have been presented in tables 1 - 3 and the concentrations are given in ng/kg (ppm).

Comments

We are aware of the possible error involved in using only one method. Using only an electron capture detector the identification and quantitative determination of the different PCB compounds is diffucult. The problem is even more complicated since the relative heights of PCB peaks change in biological tissues (Fig. 1 and 2). The gas chromatograms from some human blood and adipose tissue extracts do not resemble those of the technical products. Furthermore it is obvious that several peaks contain different compounds due to overlapping peaks. Using EC detector, concentrations can only be estimated by comparison to the response of a known amount exactly same the compound. However, the relative differences in the concentrations of PCBs of most samples is very high and the reliability of the results is acceptable.
All the examined persons are in good health. However, only the persons in group III have been under special medical observation but in spite of approximately 50 times larger PCB concentration in their blood compared with control-group I we have been unable to detect any biological effect caused by PCB in them.

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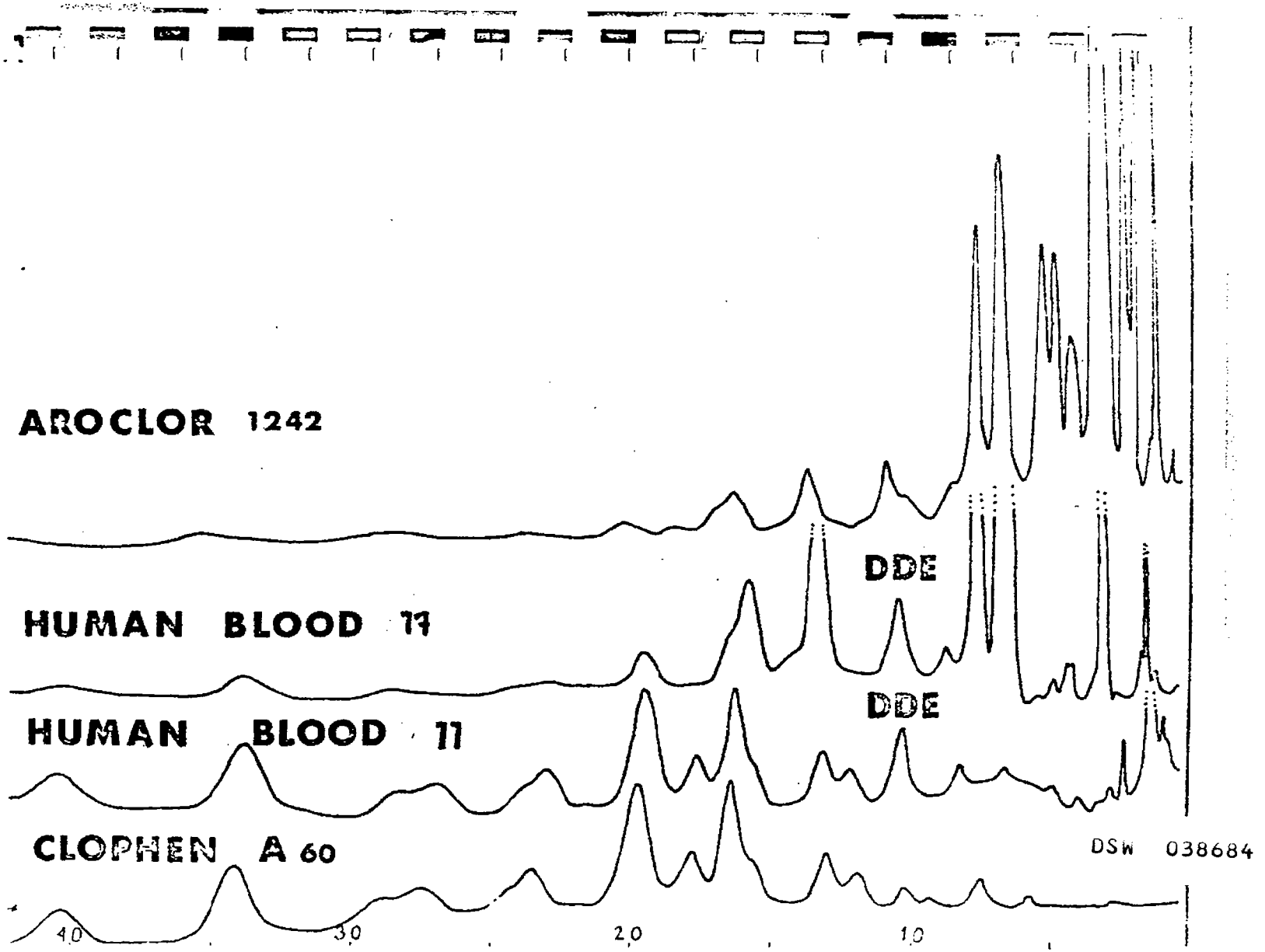
Fig. 1



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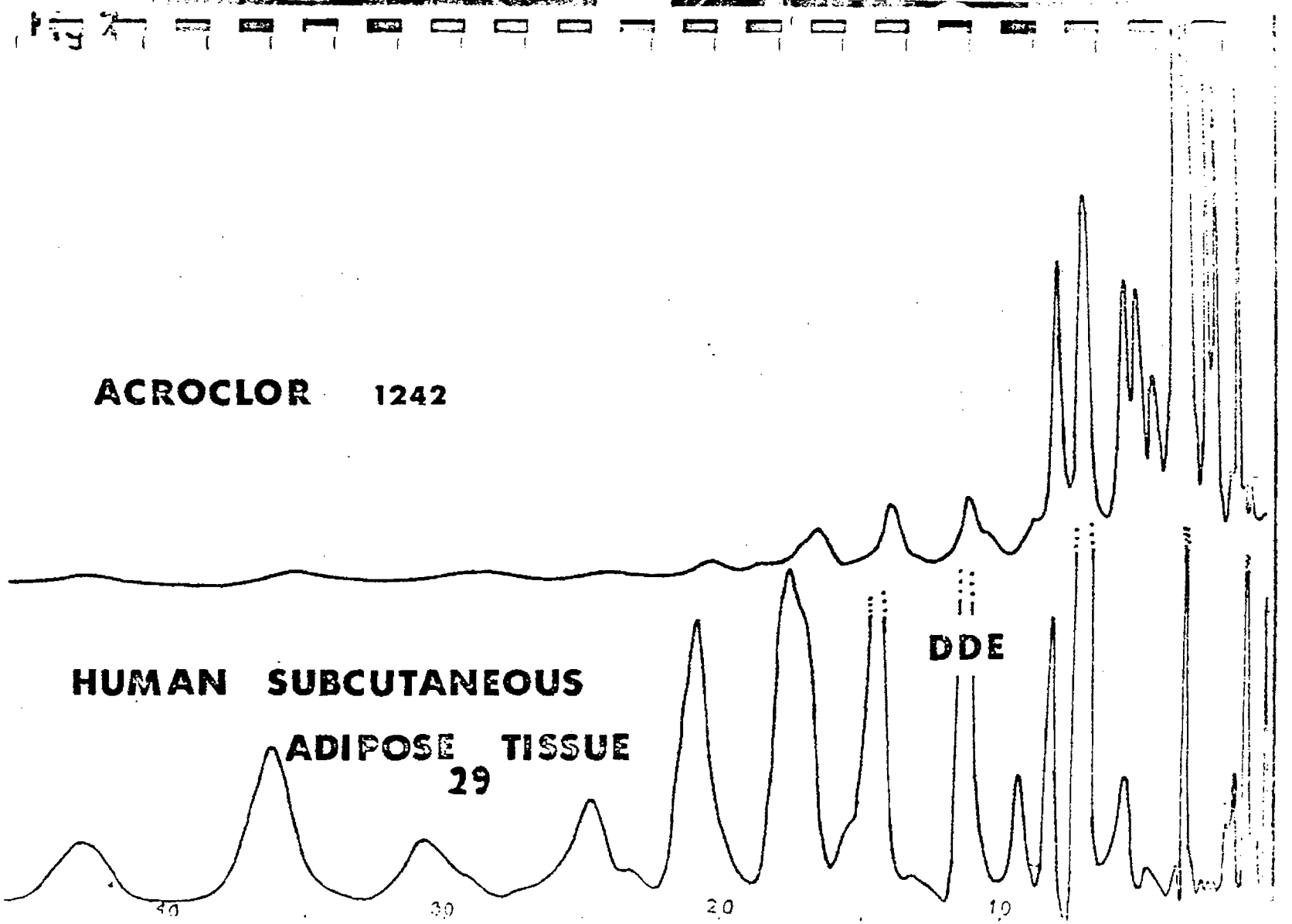

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Table 1. DDE and PCB in human blood.

Group 1. Persons without any special exposure to PCB.

No			Wet weight basis mg/kg DDE	PCB	Fat %	Fat basis mg/kg DDE	PCB	
1.	♂	Tampere	0,0046	0,012	0,13	3,6	9,9	
2.	♀	"	0,0011	0,0095	0,14	0,77	6,6	
3.	♂	Helsinki	0,0014	0,0073	0,14	1,1	5,4	
4.	♂	"	0,0016	0,0061	0,16	1,1	3,9	
5.	♀	Tampere	0,0007	0,0041	0,07	0,91	5,5	
6.	♀	"	0,0008	0,0049	0,13	0,60	3,7	
7.	♂	"	0,0018	0,011	0,15	1,2	7,0	
8.	♀	"	0,0005	0,0031	0,08	0,55	3,8	
9.	♀	"	0,0041	0,0056	0,15	2,7	3,6	
Group 2. Persons handling PCB in an analytical laboratory.								
10.	♀	Helsinki	<0,005	0,036	0,10	[1] n.c.	35	[2] 5,5
11.	♀	"	<0,005	0,040	0,12	n.c.	33	5,5
12.	♀	"	<0,005	0,046	0,10	n.c.	46	2,5
13.	♀	"	<0,005	0,050	0,08	n.c.	61	1,0
14.	♀	"	<0,005	0,063	0,09	n.c.	71	<1
15.	♀	"	<0,005	0,062	0,09	n.c.	70	<1

1) n.c. = not calculated

2) Years of reasearch work with PCB.

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Table 2. DDE and PCB in human blood.

Group 3. Workers of a capacitor factory.

No		Wet weight basis, mg/kg DDE	PCB	Fat %	Fat basis mg/kg DDE	PCB
16.	♂	0,005	0,78	0,26	1,8	305
17.	♂	0,008	1,9	0,27	3,2	700
18.	♂	<0,005	0,38	0,09	2,7	415
19.	♂	<0,005	0,49	0,10	3,0	515
20.	♀	<0,005	0,32	0,08	3,1	380
21.	♀	<0,005	0,34	0,09	4,2	400
22.	♂	0,005	0,45	0,22	2,1	205
23.	♂	<0,005	0,22	0,06	3,5	350
24.	♀	<0,005	0,11	0,09	1,9	125
25.	♂	<0,005	0,11	0,11	1,5	105
26.	♀	<0,005	0,074	0,08	2,0	100
27.	♂	<0,005	0,11	0,07	n.c.	165

n.c. = not calculated

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Table 3. DDE, DDT and PCB in human blood and subcutaneous adipose tissue.

	Blood					Adipose tissue		
	Wet weight basis, mg/kg			Fat basis mg/kg		Fat basis mg/kg		
No	DDE	PCB	Fat %	DDE	PCB	DDE	DDT	PCB
1.	0,005	0,012	0,13	3,6	9,9	2,7	1,5	2,3
2.	<0,005	0,010	0,14	0,77	6,6	0,81	0,35	1,5
28.	<0,005	0,11	0,15	2,3	76	1,2	1) n.d.	11
29.	2) n.a.	n.a.	n.a	-	-	3,8	n.d.	200
16. (atheroma)	0,005	0,78	0,26	1,8	305	3) n.c.	n.d.	285
21.	<0,005	0,34	0,09	4,2	400	3,4	n.d.	160
17.	0,008	1,9	0,27	3,2	700	2,9	n.d.	635

1) n.d. = not detected
2) n.a. = not analysed
3) n.c. = not calculated

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