PCB Conference, Stockholm, September 29, 1970.

PCB - effects on mammals.

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Already in 1899 Herxheimer described a condition that he called chloracne, which was caused by chlorinated biphenyls or chlorinated naphtalenes. Chloracne is a type of follicular pyodermatitis, e g pyongenic inflammation in the sebaceous glands of the skin. Clinical experience indicates that the capacity to produce this condition increases with chlorination of the biphenyls. Around 10 cases of fatal intoxication with chlorinated biphenyls or naphtalenes have since been described (ref. 2, 3, 4). They all showed liver atrophy and necroses. Histological examination revealed fatty degeneration, necroses and cirrhosis. All cases involved persons who handled or were exposed to these compounds in their occupations.

Animal experimentation has confirmed that chlorinated biphenyls and naphtalenes can produce liver damage of this kind. Chlorinated biphenyls and naphtalenes can be absorbed by the skin, by inhalation or by ingestion. In tables 1-3 Dr. Ulf Ahlborg (ref. 5) has summarized the published toxicological investigations in mammals involving chlorinated biphenyls and naphtalenes. The results shown on the tables indicate that the toxicity of these compounds is proportional to their degree of chlorination.

A comparison between the chlorinated naphtalenes and the biphenyls based on existing studies indicates that the biphenyls are more toxic. However, it must be emphasized that all the experiments done have been screening experiments involving technical preparations with an undefined purity, - usually mixtures of compounds having different degrees of chlorination - and that these investigations have generally focused on liver damage. Investigations of the effect on the brain for example seem to be lacking. So, in general, the present material

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permits only very limited conclusions.

It has been shown that the chlorinated biphenyls stimulate the liver microsomes as revealed by an increased metabolisation of oestrediols in the pigeon liver (ref. 9). The biphenyls seem to be about 5 times more potent in this respect than either DDT or dieldrin (ref. 9). Drinker (ref. 4) has observed a synergistic effect on the liver of carbon tetra-chloride and chlorinated biphenyls - i.e. animals exposed to chlorinated biphenyls show severe liver injury with considerable lower doses of carbon tetra-chloride.

In this connection it could be mentioned that a fatal case of biphenyl intoxication recently occurred in Finland (ref. 10). A 31-year-old man was working in an industry that used biphenyl - not chlorinated - for impregnation. The exposure level was around 100 mg/m^3, 10 times the MAC. He had been working in this industry for 11 years and suddenly developed liver insufficiency which appeared histologically as necroses, atrophy and cirrhosis. A general atrophy of the brain cortex was also found. Medical examination of the about 120 persons working in this industry revealed 3 additional cases with liver injury (biopsy) and 2 cases with pathological EEg and impaired periferal nerve function.

In order to make a toxicological evaluation of the risks involved in exposure to chlorinated biphenyls, it is necessary to have knowledge about 1) the distribution of these compounds in the body, 2) their metabolism, retention and elimination in different organs and 3) the toxicity of these substances for different cell systems and their metabolites.

With respect to the distribution, we know that there is a tendency to accumulate in body fat and in the lipid-containing tissues of the brain. That is about all we know. Our knowledge about the metabolism of these compounds is minute. From a theoretical point of view and on the basis of existing experimental data, there is reason to believe that the compounds with a low degree of chlorination should be more easily metabolised and broken down in the body than those with a high degree of chlorination. The turnover of the discrete compounds and their metabolism in different kinds of organs is unknown. The toxicity to different cell systems is also unknown

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except for the fact that liver cells can be interfered with at those concentration levels which occur in man due to industrial exposure. Liver injury also occurs early in animal intoxication experiments. However, quantitative data regarding the concentration in the liver cells at which disturbances occur is lacking at present.

Conclusion.

There is a strong suspicion that chlorinated biphenyls can be retained and accumulated in the body with chronic exposure. Present data do not permit evaluation of the risk of organ damage in the body at different doses of defined chlorinated biphenyls. There is a strong need for studies of the metabolism of chlorinated biphenyls in the body, as well as the mechanism of the toxic effect due to these substances.

DSW 202707

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REFERENCES

1. Herxheimer H., Münchener Medizin. Wochenschr. (1899) 278

2. Flinn F.B., Jarvik D.E. Proc.Soc.Exper.Biol.and Med. 35 (1936) 118

3. Greenburg L., Mayers M.R., Smith A.R. J.Ind.Hyg.Toxicol. 21 (1939) 29

4. Drinker C.K., Warren W.F., Bennet G.A. J.Ind.Hyg.Toxicol. 19 (1937) 283

5. Ahlborg U. (1969) Communication at a conference sponsored by Swedish National Board of Poison Control.

6. Miller, J.W. U.S. Publ. Health Repts. 59 (1944) 1085

7. Bennett G.A., Drinker C.K., Warren W.F. J.Ind.Hyg.Toxicol. 20 (1938) 97

8. Treon J.F., Cleveland F.P., Cappel J.W., Atchley R.W. Am. Ind. Hyg. Assoc. Quart. 17 (1956) 204

9. Risebrough R.W., Rieche P., Peakall D.B., Herman S.G., Kirven M.N. Nature 220 (1968) 1098

10. Hernberg S. (personal communication)

DSW 202708

Table 1

DERMAL TOXICITY OF CHLORINATED BIPHENYLS

Chlorine		Animal	Dose	Effect
%	atoms			
42	3	Guinea pig	34.5 mg/daily over 11 days	All animals died within 21 days. Liver damage (Miller 1944)
42	3	Rat	34.5 mg/daily over 25 days	All survived. Minor liver changes Skin affected (Miller 1944)
42	3	Rabbit	86 mg/day with 2-day intervals for 7 applications and 172 mg/day with 2-day intervals for 8 applications.	All died between 17 and 98 days. Liver damages more pronounced than in rat or guinea pig (Miller 1944)

From U. Ahlborg 1969

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Table 2

ORAL TOXICITY OF CHLORINATED BIPHENYLS

Chlorine		Animal	Dose	Effect
%	atoms			
42	3	Guinea pig	69 mg/animal 2 doses 1 week apart	Death in 11-29 days. Liver damage (Miller 1944)
42	3	Rat	139 mg/animal 25 daily doses	All animals survived Liver damages (Miller 1944)
65	7	Rat	50 mg/animal every second day	50 % dead within 35 days Severe liver damages (Bennett 1938)

From U. Ahlborg 1969

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Table 3

VAPOR EXPOSURE TOXICITY OF CHLORINATED BIPHENYLS

Chlorine		Concentration		Daily exposure time	Exposure number	Exposure period	Symptoms
%	atoms	mg/m^3	ppm	hours		days	
42	3	8.6	830	7	17	24	No effect on cats rabbits, rats and mice. Poor growth in guinea pigs (Treon 1956)
42	3	6.83	660	7	84	122	No effect on animals as above (Treon 1956)
54.3	5	5.4	410	7	83	121	Liver cell injury Increased liver weight in the rat (Treon 1956)
54.3	5	1.5	115	7	150	213	Histological changes in the liver in the rat (Treon 1956)
65	7	0.57 0.93		16 8		37-134 42-143	Advancing liver damage (Bennet 1938)

From U. Ahlborg 1969

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PCB - synthesis and radioactive labelling (— 4 Cl-atoms) x)

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The toxicity of PCB is almost unknown. Our present knowledge is based on some small scale experiments on rats exposed to PCB for not more than a month. Therefore I found it important to understand what happens to PCB once it enters the body of mammals.

Autoradiography is a specific and fast method for studying the distribution of labelled PCB. My present research deals with the discovery of possible routes for the labelling of PCB. I started with the tetrabiphenyls because they are one of the most common PCBs. The methods used here can be adapted to the synthesis of other polychlorinated biphenyls.

Some remarks on the synthesis of labelled compounds.

Small scale

The synthesis must be carried out on a small scale for two reasons - the cost and the hazards with radioactive material.

Adaptability to a closed system

The hazards with C^{14}-labelled compounds force one to use a synthesis that minimizes the exposure of the personal. Therefore, some possible good chemical solutions might be troublesome when used with C^{14}.

High yield

The expense of the starting material demands a very good yield in each step.

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Few starting compounds

The choice of starting material is very restricted. Benzene, nitrobenzen, chlorobenzene and aniline are used as starting materials.

The possible routes for synthesis of labelled PCB are often very well known. However, since many of them originated at the beginning of this century, it means that the syntheses are carried out in at least a 100 gram scale. If one uses those methods and simply scales them down, very often the synthesis will not work at all. A typical example is the industrial production of PCB, in which Cl_2 is introduced into melted biphenyl. This gives you a mixture of chlorinated biphenyls which is not wanted for toxicological studies, and too low a yield in a small scale operation. The same is true for syntheses involving diazotations.

Synthesis 1

nitrobenzene → benzidine → PCB

Nitrobenzene is reduced with zink dust and aqueous sodium hydroxide to give hydrazobenzene. It will undergo rearrangement to benzidine with hydrochloric acid. Benzidine will be acetylated by boiling for two hours in acetic acid. After cleaning, the diacetylbenzidine is chlorinated with chlorine in acetic acid solution and 3,5,3',5'-tetrachloro-N, N'-diacetylbenzidine is formed as a flocculent precipitate. It can be converted to tetrachlorobiphenyl.

Synthesis 2

Bromobenzene $\xrightarrow{\text{Step 1}}$ biphenyl $\xrightarrow{\text{Step 2}}$ PCB

Step 1

There are three synthesis from bromobenzene to biphenyl that we have studied.

I Bromobenzene is allowed to react with solid sodium for 28 hours. The yield was 29%.

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II Bromobenzene was heated with copper bronze in an ampul at 210° for 17 hours. GLC gave the composition 5:1 (bromobenzene: biphenyl).

III This is a Grignard reaction that starts with bromobenzene.

$$C_6H_5MgBr + CoCl_2 \longrightarrow C_6H_5Co\,Cl + MgBrCl$$
$$2\,C_6H_5CoCl \longrightarrow C_6H_5C_6H_5 + CoCl$$
$$CoCl + C_6H_5Br \longrightarrow CoClBr + C_6H_5$$
$$C_6H_5. \longrightarrow C_6H_6 + C_6H_5C_6H_5 + \text{polymers}$$

Using GLC we got bromobenzene : biphenyl : higher polymers : benzene 12, 5 : 18, 5 : 2 : 1. The literature reports 86% yield. (Ref. 2).

Step 2

The chlorination of biphenyl was carried out with Cl_2 in acetic acid - Cl_2 acetic acid : carbon tetrachloride (1 : 3); Cl_2 in silver sulfate and sufuric acid in carbon tetrachloride; sulfuryl chloride: silver perchlorate and chloroacetate; perchloric acid and acetic acid. Of these methods, Cl_2, silver sulfate and sulfuric acid in carbon tetrachloride gave the best result although the method is not satisfactory. After 22 hours all biphenyl was converted to monochlorobiphenyl and dichlorobiphenyl and after 13 days the end product was dichlorobiphenyl and trichlorphenyl in the proportion 1 : 3.
(Ref. 3)

Synthesis 3

1-Chloro-2, 4, dinitrobenzene → tetranitrobiphenyl

By Ullman coupling in copper bronze, 1-chloro-2, 4-dinitrobenzene is condensed to 2, 4, 2′, 4′-tetranitrobiphenyl.

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Synthesis 4

2,4-dichloroaniline → PCB

2,4-dichloroaniline is diazotised in concentrated sulfuric acid. The azocompound is put into the potassium iodide solution drop by drop. 1-iodo-2,4-dichlorobenzene formed gives 2,4,2',4'-tetrachlorobiphenyl with an Ullman coupling.

The yields from different steps have been studied with GLC measuring only the starting material and the end products. These values do not give the practical yield of the pure product and therefore only serve as a tool for discovering the best condition for a specific synthesis. The present work concerns the procedures for purifying the products and modifying the methods in order to get a better yield. At the moment, it is still too early to say which one of the syntheses that will give the best yield and will best satisfy the demands given above.

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Acknowledgment

I want to thank Dr Robert Carter and assistent Karin Öierfeldt at the Institution of Organic Chemistry at the Technical High School in Lund for synthesis 2. I also want to thank laboratory assistants Ann-Sofie Moberg and Zenon Belfer for their helpful assistance with the syntheses. and Carrol Woodworth for help in correcting the English manuscript.

References

1. Finar I L, Organic Chemistry, Longmans 1957 512.
2. Kharasch and Fields J Chem Soc 63 (1941) 2316
3. Weingarten H J Org Chem 26 (1961) 4347

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DSW 202716

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PCB - Effects on Birds

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Polychlorinated biphenyls (PCB) are widely distributed in the environment and, according to many investigators, they seem to be as ubiquitous as DDT (Risebrough et al. 1968; Koeman et al. 1969; Jensen et al. 1969; Prestt 1970; Jensen 1966). Many observations concern high levels of PCBs in the avian fauna, particularly in marine birds which are at the top of a food chain in the sea. Larger residues have been found in predatory birds than in omnivorous or herbivorous species.

There is very little work published on the avian toxicity of the PCBs. No observations concern any acute effects of these compounds in the avian fauna. As regards the sublethal effects, however, the situation may be different and there are indications that the PCBs may be responsible for certain reproduction disturbances.

There are a few works on experimental toxicology. Koeman et al. (1969) fed a diet containing 2000 ppm of Phenochlor DP6, a technical PCB mixture, to Japanese quails, all of which died between day 6 and 55 after dosing had started. Gas chromatographic analysis of the residues in liver and brain of these birds indicated that some of the more lightly chlorinated PCBs present in the original mixture, had been metabolized. Autopsy of the quails demonstrated large amounts of fluid in the pericardial sac. Hydropericard has also been observed in cockerels dosed with 400 ppm of PCB (Arochlor 1242) in their diet (Flick et al. 1965) and in Bengalese finch-

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es fed with a diet containing various amounts of Arochlor 1254 (Prestt et al. 1970).

In the latter experiment were observed signs of acute poisoning including balance disturbance at perching, paralysis in the legs and trembling with shaking wings. At the autopsy was also observed enlarged kidneys.

Flick et al. (1965) fed Arochlor 1242 to dayold cockerels (200 or 400 ppm in the diet) and reported, besides hydropericard, growth depression, abdominal and subcutaneous edema, hemorrhagic and enlarged kidneys and adrenals, a small spleen, a cream-coloured pale pancreas, decreased hemoglobin values, dermatitis and defeathering. They also discussed the similarity between the symptoms elicited by PCBs and those of the chick edema disease. Certain differences, however, exist and the possibility that the chick edema factor (today known to be a mixture of chlorinated dioxines) might be present in PCB-preparations was not supported by gas chromatographic analyses performed by McCune et al. (1962). McCune et al. (1962) also fed a chlorinated biphenyl to chicks (100 ppm) and observed symptoms similar to those of chick edema disease (hydropericard, subcutaneous and abdominal edema, hemorrhages of internal organs and enteritis).

The toxicity of PCB as compared with other chlorinated hydrocarbons, is somewhat difficult to establish, because, whereas e.g. DDT presents a relatively steep mortality curve, the curve for a PCB is much more gradual. This means that at low dose rates. PCB may have a comparatively greater toxicity than similar amounts of DDT, but at higher dose rated the toxicity of DDT is considerably higher. In the experiments of Prestt et al. (1970) with Bengalese finches, the estimated dose rate for 50 % mortality at 56 days was 254 mg/kg/day. At low doses, PCB was more toxic than DDT; at higher doses PCB was about 10 times less toxic than DDT.

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Prestt et al. (1970) also gave some values of the concentrations of PCB in some organs of the birds. One bird, killed after 8 weeks daily feeding with 367 µg of PCB, showed 14 ppm in the brain, 25 ppm in the liver, 574 ppm in fat and 61 ppm as a mean in the rest of the body. 9 % of the ingested amount remained in the body, and was stored mainly in the depot and organ fa . Some of the birds were starved prior to analysis. Starvation resulted in a redistribution of PCB with an increase of the brain concentration.

The population declines of many avian species in Europe and North America has been attributed to the use of chlorinated hydrocarbons. This decline has been traceable not to the death of adults, but to a drastic drop in reproduction. The symptoms observed in connection with this decline have included late breeding, failure to lay eggs, a remarkable thinning of the shells and much breakage of the eggs that are laid, eating of broken eggs by the parents, failure to lay more eggs after earlier clutches have been lost, reduced clutch size and a high mortality of the embryos and among fledglings. The regions of population decline coincide with areas where chlorinated hydrocarbons are widely applied.

The pato-physiological mechanisms behind these symptoms are uncertain, but may to some parts be attributed to the capacity of the chlorinated hydrocarbons to disturb the hormone balance and the function of enzymes.

It has been demonstrated in pigeons, that PCBs, like DDT and DDE, have the capacity to induce steroid hydroxylating liver enzymes to increase their breakdown of estradiol(Risebrogh et al. 1968).

The PCBs are more powerful inducers of these enzymes than DDT or DDE. The breakdown of estradiol in pigeon liver was increased 2.5 times by DDE, 3.5 times by DDT and 5.5 times by PCB, in spite of that only half as much

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PCB as DDT or DDE was used (Risebrough et al. 1968).

The late breeding, the delayed egg-laying and the failure to lay again after early loss of eggs could be explained in this way because estradiol is involved in the stimulation and maintenance of the sex organs and breeding behaviour. A delayed laying may be very serious for certain species because this reduces the chances for the young to get optimum of feed and consequently reduces the optimal chance for survival. Concerning this risk it appears that the PCBs are greater threats to birds than DDT.

The failure to lay at all could also be caused by decreased hormone concentrations resulting from hepatic enzyme induction or apparent failure to lay could be caused by early breakage and eating of eggs. A reduced clutch size could also be caused by the breakage of eggs.

Since about 1947, there has been a sharp decrease in the thickness of the egg shell of certain predatory birds whose populations were decreasing (Risebrough 1968 and others). Thin eggshells were found in avian species who accumulated high concentrations of chlorinated hydrocarbons. In uncontaminated populations of these species the production of eggshells was normal. Extremely thin eggshells have been found in the brown pelican in California where the average percentage thinning was 53 % and the extreme was 95 %. In North American peregrines, contaminated with DDT as well as PCBs, a decrease in eggshell thickness of 34 % from the mean has been demonstrated. The reduction in eggshell thickness increases the chances of egg breakage. Water retention, which affects a successful hatching, might also be impaired. The PCBs cause thinning of the eggshells but this thinning seems not to be as effective as that caused by DDT or its metabolites.

The formation of thin egg-shells could be explained by two

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different mechanisms. The deposition of calcium in the medullary bone, a storage necessary for the egg-shell production, is under control of estradiol. A decreased concentration of this hormone caused by hepatic enzyme induction as mentioned above, might influence the thickness of the egg-shell. However, it seems unlikely that a reduction of the calcium reserve in the medullary bone, alone could be responsible for the marked thinnings often observed. The birds could compensate this lack by drawing calcium from the skeleton. Furthermore, birds on a very low calcium diet have been found tocease egg-laying rather than laying thin-shelled eggs. An acute hypocalcemia might also result in flight disturbances since the muscle- and the nervous systems would be affected.

Another mechanism therefore seems to play the major role in the thinning of the shells. Carbonic anhydrase is necessary to supply the carbonate ions required for calcium deposition in the shell. Several investigations have shown a lower concentration of carbonic anhydrase in shell glands producing soft-shelled eggs or no eggs than in glands producing normal eggs. Inhibition of this enzyme by DDT and DDE has been demonstrated and the close similarity between these compounds suggests that PCB might have a similar effect. It's interesting to note that no threshold value has been found for the effect of DDE on egg-shell thickness. Very small doses will cause some thinning of the shell.

Eggs from several species of birds have been demonstrated to contain large amounts of PCB (Risebrough 1968; 1970). This might contribute to explain the decreased hatchability. Chick embryo experiments have also shown that injection of small amounts of PCB in the eggs will cause a high mortality. Certain signs also indicate teratogenic effects. (Mc Laughling et al. 1963).

Finally, another interesting aspect of these compounds should

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be mentioned. It has been demonstrated in flies that various PCBs significantly may increase the toxic effects of dieldrin and DDT (Lichtenstein et al. 1969). No information has been found on such an interaction in the tissues of higher animals. However, the potential risks should not be disregarded in view of the synchronous occurrence of these compounds in many populations.

DSW 202722

References

Flick, D.F., R.G. O'Dell and V.A. Childs (1965). Studies of the chick edema disease. 3. Similarity of symptoms produced by feeding chlorinated biphenyl. Poult. Sci. 44, 1460 - 1465.

Jensen, S. (1966) Report of a new chemical hazard. New Scient. 32, 612.

Jensen, S., A.G. Johuds, M. Olsson and G. Otterlind (1969). DDT and PCB in marine animals from Swedish waters. Nature, Lond., 224, 247 - 250.

Koeman, J.H., M.C. Ten Noever de Brauw and R.H. de Vox (1969). Chlorinated biphenyls in fish, mussels and birds from the river Rhine and the Netherlands coastal area. Nature, Lond., 221, 1126 - 1128.

Lichtenstein, E.P., K.R. Schulz, T.W. Fuhremann and T.T. Liang (1969). Biological interaction between plasticizers and insecticides. J. Econ. Entomol. 62, 761 - 765.

McCune, E.L., J.E. Savage and B.L. O'Dell (1962). Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Poultry sci., 41, 295 - 299.

McLaughling, Jr. J., J.P. Marliac, M.J. Verrett, M.K. Mutchler and O.G. Fitzhugh (1963). Toxicology and Appl. Pharmacol., 5, 760 - 766.

Prestt, J., D.J. Jefferies and N.W. Moore (1970). Polychlorinated biphenyls in wild birds in Britain and their avian toxicity. Environ. Pollut., 1, 3 - 26.

Risebrough, R.W., P. Rieche, D.B. Peckall, S.G. Kerman and M.N. Kirven (1968). Polychlorinated biphenyls in the global ecosystem. Nature, Lond., 220, 1098 - 1102.

DSW 202723

STLCOPCB4052887

PCB - synthesis and radioactive labelling ($\geq$ 4 Cl-atoms).

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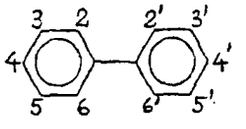
Polychlorinated biphenyls are technically prepared by chlorination of biphenyl (1.) at 70 - 150°C, using iron filings or iron(III)chloride as catalysts. The chlorination is interrupted at the desired chlorine content, depending upon the principal use of the product. The commercial products generally contain from 50 to 65 % chlorine, which roughly corresponds to 4 - 8 chlorine atoms per molecule.



(1)

In spite of the technical importance of these products little if any information is available on their composition in terms of defined structures of its components. The purposes of the present, so far preliminary investigation are thus twofold:

a) Synthesis of symmetrical and unsymmetrical polychlorobiphenyls of known structures for comparison with major components of technical PCB

b) Synthesis of one ore two C^{14}- labelled polychlorobiphenyls of known structures, preferably containing six or more chlorine atoms.

The synthetic work mentioned under a) will serve also as model experiments for synthesis of labelled compounds. With due regard to the commercial availability of aniline and nitrobenzene, uniformly labelled with C^{14} (30 - 50 mC/mmol), two routes to biphenyls which both involve diazotisation of chlorinated anilines have been chosen for preliminary studies - the Ullman reaction and the Gomberg-Bachmann diazo reaction.

To permit the use of the Ullman reaction[1], a phenyldiazonium salt is transformed to a iodobenzene derivative which in the presence of

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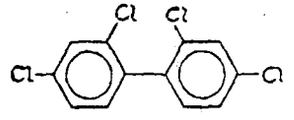
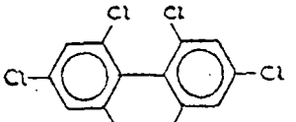
copper powder reacts at high temperatures (200 - 250°C) with formation of a symmetrically substituted biphenyl. Under similar conditions, a mixture of two different iodided gives one unsymmetrical and two symmetrical biphenyls, which can often be separated by chromatography. The preparative value of the Ullman reaction is, however, sometimes reduced by the occurrence of several side reactions which differ in relative importance already among the ortho-, meta- and para-chloro-iodobenzenes.[2] 2,2',4,4'-Tetrachlorobiphenyl (2) (m.p. 83°) and 2,2',4,4',6,6'-hexachlorobiphenyl (3) (m.p. 112°) were prepared in about 50 % yields from the readily accessible dichloro- and trichloro-anilines via the iodides already in 1904 by Ullmann[3]. A comparison by means of GLC of the hexachlorobiphenyl 3 with technical PCB (Arochlor 50) excludes its occurrence as a main component in the technical product. A similar comparison performed with a crude product containing the tetra-chloroderivative 2 does not exclude its occurrence at least as a minor component.



2



3

At the technical chlorination of biphenyl, the first two chlorine atoms can tentatively be assumed to enter positions ortho or para to the biphenyl linkage (2, 2', 4 or 4'). The following four atoms should enter mainly available free positions ortho and para to the already introduced chlorines. Hence, 2,2',4,4',5,5'-hexachlorobiphenyl (4) might be assumed to occur in a highly chlorinated technical PCB. This compound should be available through the Ullmann reaction performed with 2,4,5-trichloro-iodobenzene (5). Studies on such a coupling reaction are under way. Preliminary results of GLC experiments, as performed by dr. S. Jensen,

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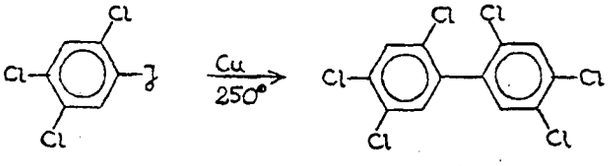
strongly indicate that the Ullmann main product is also a main component of technical PCB. It gives a retention time corresponding to peak 8 of Arochlor 50.



5 4

The Gomberg-Bachmann diazo reaction[4] for synthesis of unsymmetrical biaryls involves diazotisation of an aniline derivative followed by the addition with rapid stirring of alkali and excess of a coupling partner, in the present case a di-, tri- or tetrachlorobenzene. Preferably the latter sould be a liquid at the reaction temperature (0 - 30°C) to permit facile coupling without the addition of interfering solvent. The coupling step proceeds in the organic phase via the phenyldiazohydroxide (or its equivalent) 6 as exemplified in Scheme I. The reaction gives a mixture of biphenyls, except in cases were benzene or a 1,4-disubstituted benzene derivative is used as coupling partners. Fair amounts of low-molecular by-products and resinous material are generally observed. Due to the side reactions, which will not be discussed in detail, the total yield of biphenyls is only low to moderate. The diazo reaction has so far not been reported in connection with synthesis of polychlorobiphenyls.

In the present work the usefulness of the Gomberg-Bachmann reaction has been evaluated as represented in Scheme I; the total yield of biphenyls was about 25 %.

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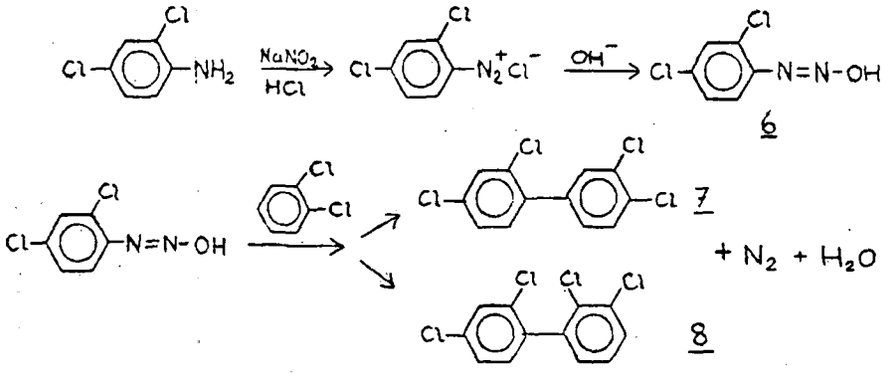
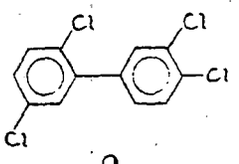
Scheme I



GLC of the purified product indicated the presence in about equal amounts of the two expected main components, 7 and 8. So far, a compound m.p. 125-126° has been isolated in a pure state and formulated as 2,3,4,4'-tetrachlorobiphenyl (7) from its spectral properties.

Similarly, the reaction of 2,5-dichlorophenyldiazonium hydroxide with 1,2-dichlorobenzene has given a product shown by GLC to contain some minor components and a major compound, the latter tentatively formulated as 2,3',4',5-tetrachlorobenzene (9) although the isomeric structure 2,2',3',5-tetrachlorobiphenyl (10) cannot be excluded.



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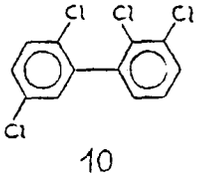

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A GLC comparison with Arochlor 30, performed by Dr. S.Jensen, makes probable the occurrence of the isomers 7 and 9 (or 10) in the technical product (fig 1 and 2).

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From model experiments on a micro scale on partial chlorination of aniline and on the reaction sequences of Scheme I, it seems possible to reach a mixture of biphenyls 7 and 8, C^{14}-labelled in the left ring with a total activity 10-20 % of the activity of the starting material, aniline-C^{14}. The isomers 7 and 8 are well separated by GLC.

The diazo reaction has so far not been applied to chloroanilines or to coupling partners containing three ore more chlorine atoms. Likewise, the use of the Ullmann reaction on a micro scale has not been tried.

References:

1. Fanta,P.E. Chem.Rev. 38 (1946) 137
2. Ullmann,F. Liebigs Ann. der Chemie 332 (1904) 38
3. Nilsson,M. Acta Chem.Scand. 12 (1958) 537
4. Bachmann,W.E. and Hoffmann, R.A. Organic Reactions 2 (1944) 225

DSW 202728

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Fig 1.

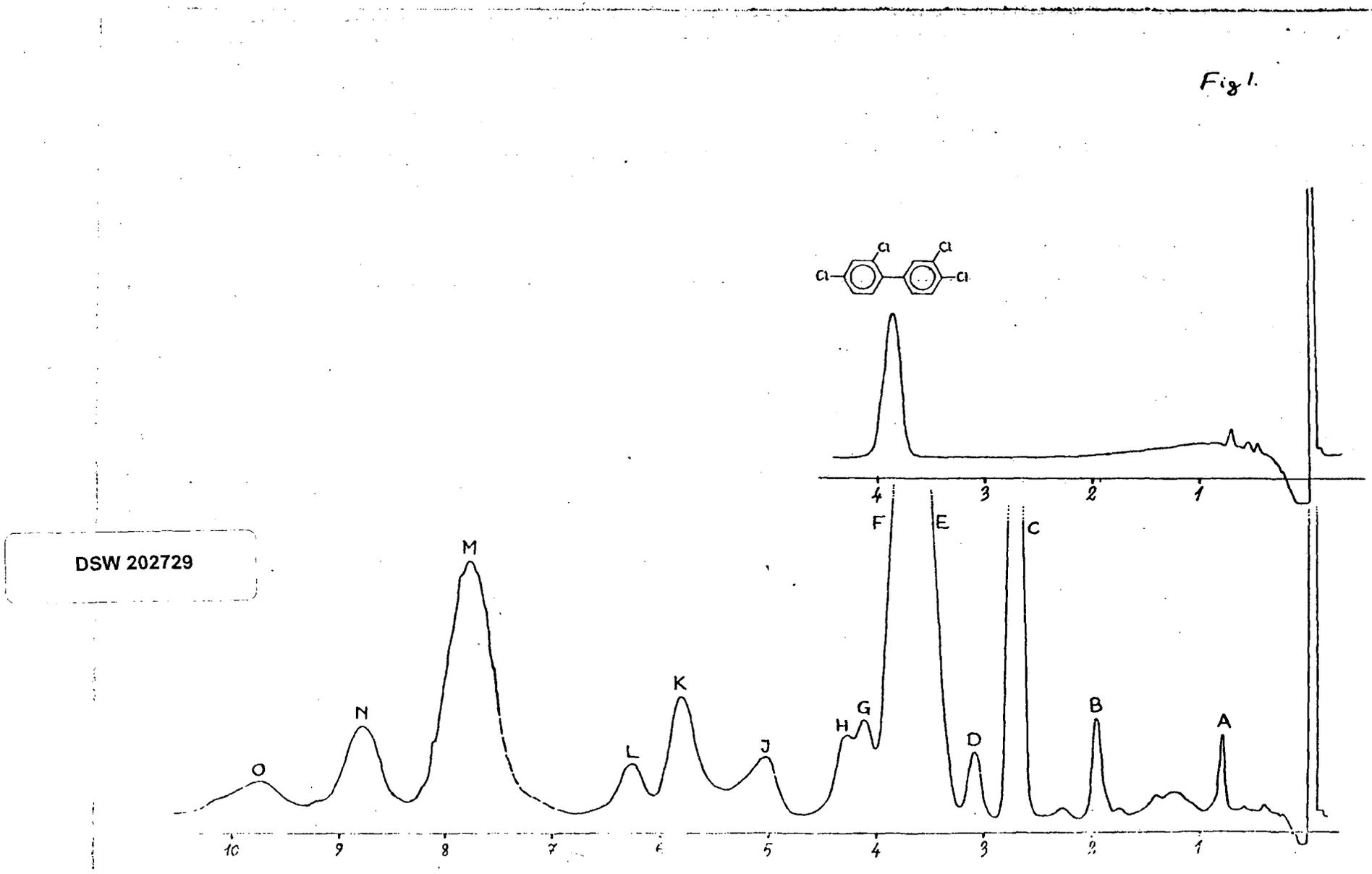

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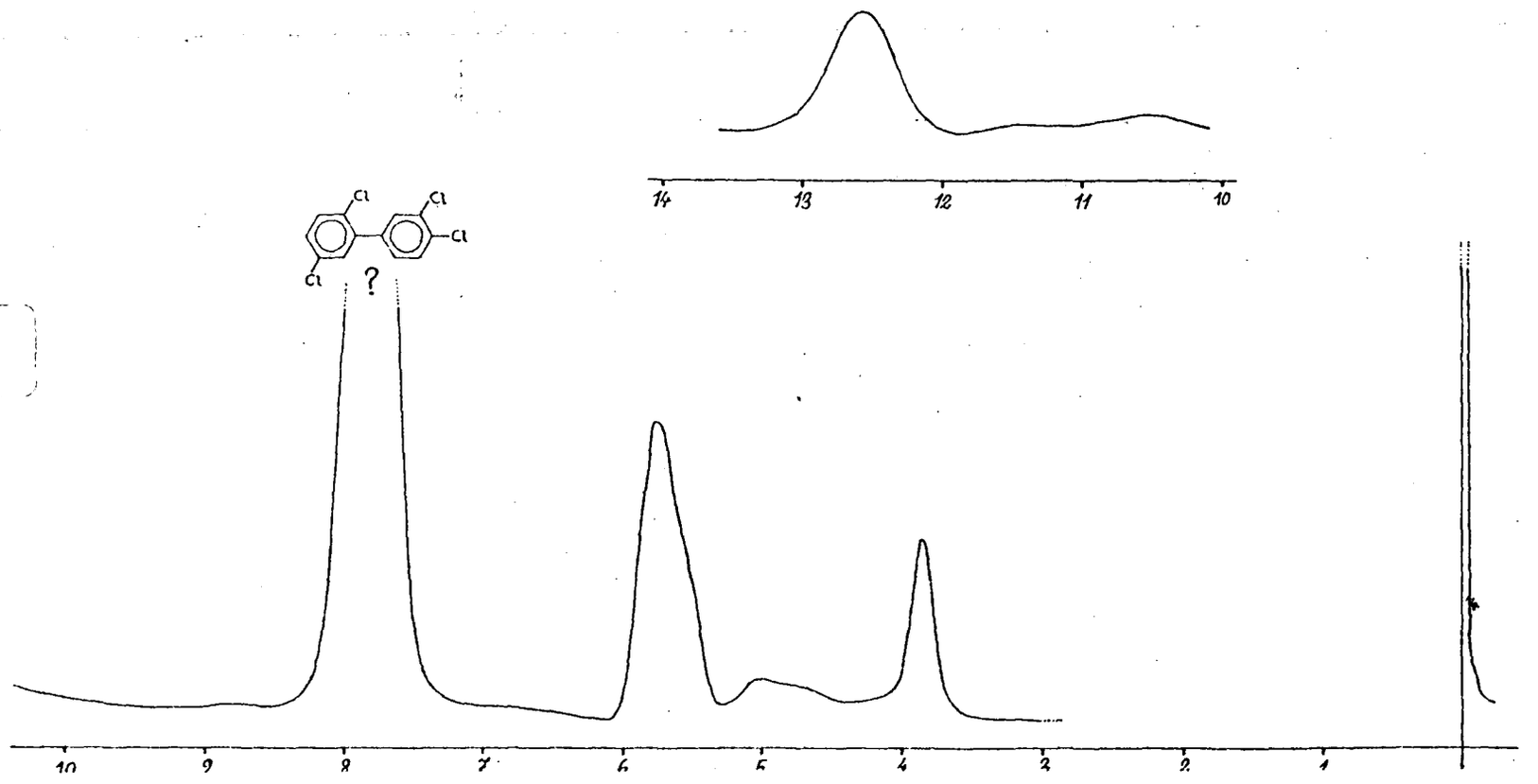
Fig 2.



DSW 202730

STLCOPCB4052894

PCB - Indications of effects on salmon

by

S. Jensen[1/], N. Johansson[2/] and M. Olsson[3/]

1/ Natl. Environment Protection Board.

2/ Salmon Research Institute.

3/ Swedish Museum of Natural History.

Information concerning the effect of PCB residues in fish, or the possible hazard posed by such residues to fish, is presently lacking in the literature. Studies of the effect of the chemical on salmon and salmon reproduction has recently started at the Salmon Research Institute.

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Effects on the liver in young salmon.

"Pale liver" is a frequently appearing pathological condition among young salmon in Swedish salmon rearing stations. The condition is related to a high degree of stainable fat in the organ, as revealed by the Sudan Black B technique (JOHANSSON, 1965). There are two commonly accepted theories about the cause of this type of "fatty livers": one is based on the probably existence of a toxic substance in the organism, the other postulates a degenerative action of imbalanced diets on hepatocytes.

MILLER (1944) exposed guinea pigs, rats and rabbits to chlorinated diphenyl by injection, applications to the skin and by feeding. Liver damage - fatty degeneration, atrophy of the centrolobular cells and perinuclear basophilic granulation were present in the test groups. Consequently the primary objectives of our investigations were to study the accumulation of PCB in salmon and whether chronic exposure to sublethal levels of the chemical has a direct pathological effect on the liver.

A group of 80 one-year old salmon (average weight 18 g), raised at the institute, were used for the first test. Forty of these were exposed to a constant flow of a commercial PCB product (average 5 Cl per molecule) for two month in a concentration of 1 ppb. The rest of the fish served as control. Both groups of fish were held in square tanks made of fiber glass (bottom area 0.9 m^2). The water flow was 10 lit/min and the water temperature 10° C $\pm$ 1. Samples were taken before the experiment began, and after 8, 12, 15, 18 and 50 days of exposure and were sent to the University of Stockholm (JENSEN) for residue analyses. At the same time samples were taken for histological examination. Livers were fixed in Bouin's Fixative mod. Hollande and 10 % Neutral Buffered Formalin pH 5.7. No mortality occured during exposure of fish to PCB.

Residue analyses showed, that the concentrations of PCB in fish from the test group and from the control group were of about the same size. (Befor the experiment began: 21.1 ppm in liver on fat basis. After 50 days of exposure: test group average 60.4 ppm, controls average 42.2 ppm in liver on fat basis).

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The histological examination of the material is not finished in all details, but hepatic fat occurred in practically all fishes in the test group and in the controls as well. However, in the controls the condition was possibly more slight or moderate in degree compared with the test group. Degenerating liver cells, some of them with vacuolated nuclei appeared more frequently in the test group than in the controls. Acid-fast pigment of ceroid type was noted for some fishes.

The concentrations of PCB residues in pretreatment samples and in the control group threw suspicion on occurrence of PCB-pollution of the aquatic environment. Later analyses of fish from the institute have confirmed this. To start new experiments it was necessary to get fish without basic levels of PCB-residues. After some efforts this have been done and a second test could start a few weeks ago.

Effects on salmon reproduction.

The young salmon reared at the Salmon Research Institute originates from ova taken from females grown up in the sea as well as from females reared up to maturity in fresh water at the institute. The latter females used for spawning usually have been kept in fresh water for three to eight years.

Since 1965 heavy losses of eggs originating from the females kept in fresh water have been noted, while eggs from females grown up in the sea have shown normal survival (Fig. 2A). All kinds of eggs have been incubated and handled in the same manner.

Eggs and fry have been examined for known diseases every year since 1966, but, with the exception of slight outbreaks of yolk-sac strangulation in some lots of fry in the years 1967 and 1968, we have never been able to make any true diagnosis. As most of the ova lost are supposed to be unfertilized, examinations of the gonads of parent males and females have been done. In the male gonads no structural abnormalities were found by studying stained sections in light microscope. The motility of sperm cells as well as the volume of the milt was normal. In the females, the maturing of the ovaries, expressed in egg diameter, egg number and weight of the ovary in percent of the total weight, seems to be normal compared with females grown up in the sea (JOHANSSON, to be published).

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In view of the differences in viability of ova between the two types of parent females, and in the light of the fact that PCB pollution of the aquatic environment had been noticed, a study of the effect of PCB on reproduction in salmon was of immediate interest.

Primary it was necessary to find out whether high losses of eggs from certain females could be related to a high level of PCB residue. Nine females of different ages, (8+:1,[1] 7+:2, 6+:1, 5+:2, 4+:1 and 3+:2) and reared up to maturity in fresh water were selected for this study. Two females (ages 2.4+[2] and 2.3+), grown up in the sea served as controls (See table 1). One of these (W. 156/69) was from the river Lule älv, the other (W.D.) from the river Dalälven.

Eggs were stripped from the females on October 28 to November 10, and fertilized by milt from hatchery reared males. After water hardening, the eggs were incubated at the institute. The number of eggs in each lot varied between 2000 and 4000. The eggportion from female number 156/69 was divided into two equal lots. The one was incubated in the Heden station at the river Lule älv, the other one at the Salmon Research Institute at the river Dalälven.

Samples of eggs were taken two days after stripping and were sent to the Natl. Environment Protection Board-Special Laboratory, Uppsala for residue analyses. In some cases (see table 1) samples also were taken from eyed eggs.

The results of residue analyses are given in table 1. As the proportion of lipids present in salmonids and salmonid eggs is very variable, (the range for Salmon beeing 0.35-14 %) the concentrations are based on extractable fat.

a. Eggs from females grown up in the sea: PCB residues of 7.7 and 9.2 were noted for the samples taken two days after stripping. For female number 156/6 the concentration increased from 7.7 ppm to 14.0 ppm (6.3 ppm) during the period November to February in the lot of eggs which had been incubated at the river Lule älv, while the corresponding increase for the lot incubated at the river Dalälven were 7.7 to 19.8 ppm(12.2 ppm). The amount of fat in the ova was about the same in both lots.

1) 8+ indicates eight years and one summer in fresh water.

2) 2.4+ indicates two years in fresh water, four years in the sea and one summer.

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b. Eggs from females reared in fresh water: The PCB residues found varied from 14.4 to 34.0 ppm when analyzed two days after stripping. Thus, the PCB residues were higher in eggs from females reared in fresh water than those in eggs from females grown up in the sea. On the other hand higher concentrations of DDT were noted for the "wild" females. The total DDT values in eggs from females number 61-13, 63-01 and 65-02 are mainly due to DDE.

As shown in fig. 1, the losses of ova during incubation varies from 13 to 100 % and are smallest in the lots from "wild" females. High losses correspond to high concentrations of PCB residues. The main losses are due to unfertilized eggs or to mortalities in an early stage of development. There are no extraordinary mortalities to be found in the yolk-sac stage.

In spite of the small material used, this preliminary study indicates a possible relationship between PCB in salmon eggs and egg loss. In order to find out if this relation is true, new experiments have been prepared for the season 1970/71. For these tests females exposed to known concentrations of PCB will be used for stripping of eggs.

Since there are pronounced variations in the viability of ova and fry in different Swedish salmon rearing stations and since increased losses of eggs have been noted for some stations in the last years, also a survey of the contamination of our east coast rivers would be of great value.

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Female from family number	Female age	Organochlorine compounds in ova								% fat		% mortality
		Σ DDT fat basis		DDT fat basis		PCB fat basis		PCB fresh w. basis				
		1	2	1	2	1	2	1	2	1	2	
62-77	7+.	n.d.	n.a.	n.d.	n.a.	34.0	n.a.	1.90	n.a.	5.4	n.a.	100
65-310	4+.	n.d.	n.d.	n.d.	n.d.	27.6	30.0	1.9	1.6	6.9	5.3	99
64-10	5+.	n.d.	n.d.	n.d.	n.d.	20.2	25.0	1.2	1.0	5.9	4.2	98
63-17	6+.	n.d.	n.d.	n.d.	n.d.	26.9	34.0	1.6	1.9	5.9	5.5	82
61-52	8+.	n.d.	n.a.	n.d.	n.a.	31.7	n.a.	1.7	n.a.	5.4	n.a.	70
61-13	7+.	7.2	n.a.	n.d.	n.a.	17.5	n.a.	1.0	n.a.	5.7	n.a.	70
66-302	3+.	n.d.	n.d.	n.d.	n.d.	14.4	23.0	0.6	0.9	4.4	3.8	53
63-01	5+.	6.2	n.a.	n.d.	n.a.	15.8	n.a.	0.9	n.a.	5.8	n.a.	48
65-02	3+.	5.7	n.a.	n.d.	n.a.	15.2	n.a.	0.6	n.a.	4.2	n.a.	46
W. 156/69	2.4+	12.5	11.3	9.5	2.8	7.7	19.8	0.6	1.2	7.9	5.9	34
W. 156/69	2.4+	12.5	12.2	9.5	4.5	7.7	14.0	0.6	0.8	7.9	5.7	29
W.	2.3+	33.5	n.a.	11.6	n.a.	9.2	n.a.	0.4	n.a.	3.8	n.a.	16

Table 1. Concentrations of organchlorine compounds (ppm) in salmon ova and mortalities of eggs and sac fry from fertilization to the feeding stage; n.d., not detectable; n.a., not analysed; 1 = analysed two days after stripping; 2 = analysed from eyed eggs; W = wild female.

Σ DDT = DDT + 1.11 x (DDD + DDE).

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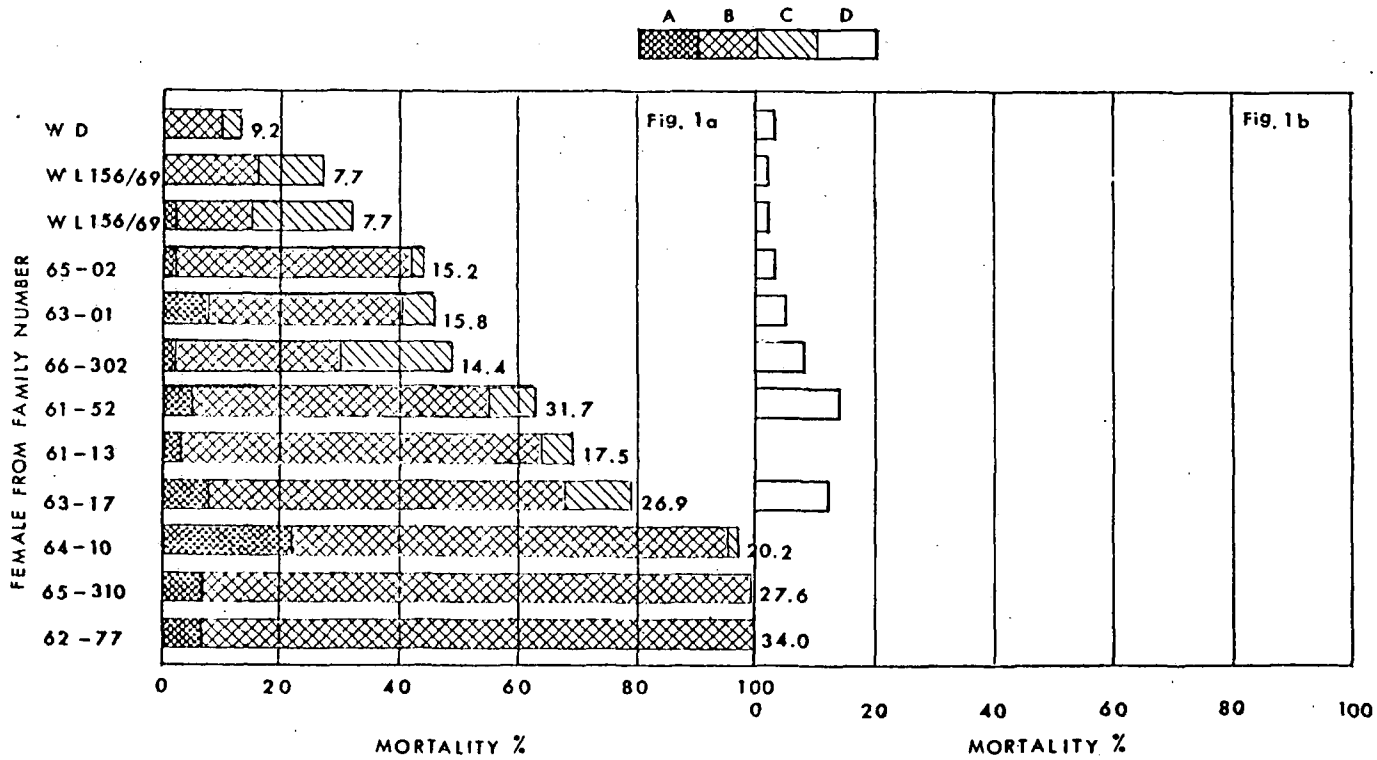

Fig. 1. Percentage of mortality during incubation (fig. 1 a) and in the yolk-sac stage (fig. 1 b); ova dead at the beginning of incubation (A); dead ova without developed embryos (B); dead ova with developed embryos (C); dead sac fry (D). W L and W D indicates females grown up in the sea. To the right of each bar is recorded the concentration of PCB residue in ova two days after stripping (ppm in fat).

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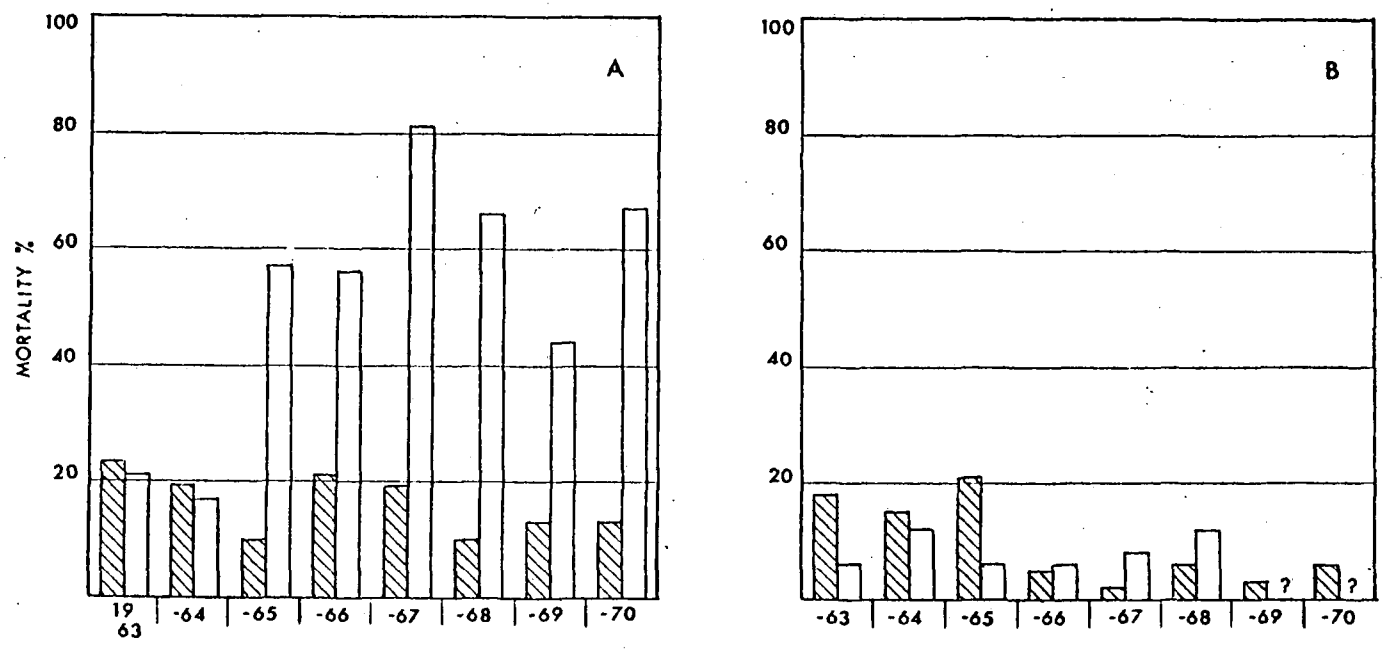

Fig. 2. Average losses of ova from fertilization to hatching (A.) and mortalities during the yolk-sac stage (B.) 1963 - 1970. Open bars = eggs and fry from females in fresh water; shaded bars = eggs and fry from females grown up in the sea.

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REFERENCES

Carlin, B., Svensson, K.M., 1965. Redogörelse för uppfödningen vid Älvkarleö under 1964. Swedish Salm. Res. Inst.-Report 1/1965.

Ghittino, P., Ceretto, F., 1962. Studio Solla Eziopatogenesi dell'epatoma della Trota Iridea di Allevamento. Estratto da Tumori 48(6), 393-409. 1962.

Johansson, N., 1965. Histological Studies of the Livers from 1 1/2-year to 2-year old Salmon (Salmo salar L.) raised on Different Diets. Swedish Salm. Res. Inst.-Report 9/1965.

Johansson, N., The Sexual Maturing of Salmon Females reared in Fresh Water and Fed Different Diets. (To be published).

Miller, J.W., 1944. Pathological Changes in Animals exposed to a commercial Chlorinated diphenyl. Publ. Health Reports. 59(33). 1085-1093. 1944.

Rucker, R.R., Yasutake, W.T., Wolf, H., 1961. Trout Hepatoma- a Preliminary Report. Prog. Fish-Cult. 23(3) 1961.

DSW 202739

STLCOPCB4052903

PCB Conference, Stockholm, September 29, 1970.

PCB in Swedish wildlife
A preliminary report

S Jensen, A G Johnels, M Odsjö, M Olsson and G Otterlind

In 1966 Jensen identified polychlorinated biphenyls (PCB) in samples of fish and birds and also found indications that these compounds might appear as contaminations in the ecosystems. Since then it has been demonstrated that the PCBs are widely dispersed in the global ecosystem (Risebrough et al. 1968). Studies have shown that they are also distributed in Britain (Prestt et al. 1970), in the coastal areas of the Netherlands (Koeman et al. 1969), and in the marine waters along the Swedish coast (Jensen et al. 1969).

The treatment of the material and the analytical method has been described previously (Jensen et al. 1969). The material has been collected at localities, the positions of which are indicated in fig 1. The values based on extractable fat are of particular interest from an ecological point of view (Jensen et al. 1969). Fat is important in the transport of energy and as a carrier of residues between the species in the food web. Furthermore, there is a seasonal variation in fat content within the populations. The difference between the greatest and the smallest residue level in the extractable fat of individuals of one species, at a given time in a given place, is less than in fresh tissue. The figures therefore are based largely on resudue levels in extractable fat.

Terrestrial

Adult specimens of starling (Sturnus vulgaris) have been sampled on their arrival at breeding areas where later also juveniles have been collected. The sampling has been performed in three years, 1967-69. Pectoral muscle has been removed and each specimen has been analysed individually. The results are presented in fig. 2. Locality 1, Krankesjön, is situated

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in typical farmland in the southernmost part of Sweden. Locality 2, Kvismaren, also is dominated by cultivated areas. As in the case of locality 1 the bedrocks here is of cambro-silurian origin. Locality 3, Kilsbergen, is situated only 20 kilometres to the west of number 2 but the area is dominated by coniferous forests and the bedrock is granite. Locality 4, Enånger, is within the area of coniferous forest typical of North Scandinavia. Although there is a variation in the levels between the years, it can be seen from the diagram (fig 2) that they are, as a rule, lower in the juveniles than in the adults. Although the localities are widely distributed and differ markedly concerning natural conditions as well as influence of human activities the levels are roughly the same within the groups of juveniles and adults. The adults have spent the winter in western Europe (Great Britain, the Netherlands, Belgium and France). It is known that on return from these areas they contain some dieldrine which has been acquired within their winter quarters. If the same process takes place in the case of PCB is not certain. The lower content of PCB in the juveniles than in the adults may be the result of a slow accumulation process.

The levels of total DDT (DDT and metabolites of this compound) and of PCB in pectoral muscle and eggs of some Swedish birds are listed in table 1. With the exception of a few adult fieldfares with an exceptionally high level of DDT the predatory birds are, as is well known, generally on a higher level than other birds in the case of both types of compounds. The levels are higher in the adults and as a rule also the ratio between DDT and PCB is higher in the adults than in the juveniles.

Fresh water

Samples of pike have been collected in the years 1967, 1968 and 1969 in lake Bolmen (fig. 1, nr 5) in the southern part of Sweden and for some of the years in lake Storvindeln (fig 1, nr 8) in the northern part of Sweden. Some specimens are

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available also from the lakes Roxen and Ymsen (fig 1, nrs 6 and 7) in the south central part of Sweden. The results from lake Bolmen indicate that considerable changes may occur between the years. It seems, however, that the ratio between DDT and PCB is fairly constant. Bolmen is an ologotrophic lake and so is Storvindeln. This lake is very remote from industrial influence, at least within the borders of Sweden, but nevertheless the levels of PCB seem to be roughly comparable between these two lakes. The lakes Ymsen and Roxen are both highly eutrophic. However, Ymsen is located in a farmland area while Roxen also has a strong influence from densely populated and industrialized areas. This may serve to explain the great difference in PCB-content, Roxen being very high.

Marine

In table 3 material of the following species is included: herring (Clupea harengus), Dog fish (Squalus acanthias), Salmon (Salmo salar), Grey seal (Halichoerus grypus), Common seal (Phoca vitulina) and ringed seal (Pusa hispida), guillemot (Uria aalge), white-tailed eagle (Haliaetus albicilla). A few samples of separated fish oil were also analysed. Two of the samples from the west coast were from the Norway pout (Boreogadus esmarki) and one from herring. The single oil sample from the Baltic is herring oil which was unseparated.

In fish axial muscle tissue was analysed. Most of the samples from the seals were taken from the blubber in the tail. In the white-tailed eagle samples were taken from both the pectoral muscle and the brain. All the eagles were found dead and in some cases in a state of decay.

DDE is the principal metabolite of DDT found. In the case of seals from the Archipelago of Stockholm and in salmon from the Baltic proper relatively large amounts of DDD were found (about 40 % DDD of total DDT).

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The mean figures in table 3 are calculated from the average value of each locality within the regions "Swedish West Coast", "Baltic Sea" including the sound between Sweden and Denmark, "the Archipelago of Stockholm" and the "Gulf of Bothnia". The residue values are based on hexane extractable fat. Most of the material including figures on fresh tissue basis have been published earlier (Jensen et al. 1969).

The samples of herring from the Baltic proper, the Archipelago of Stockholm and the Gulf of Bothnia had all larger residues of DDT-compounds and PCB than the herring samples from the West Coast. Juvenile herring from coastal waters of the Netherlands contained DDE-residues in fresh tissue with an upper level of the same magnitude as in herring from the Baltic Sea area (Koeman et al. 1967). Residues of PCB and DDT-compounds in one sample of fish oil from the south part of the Baltic were about 5-10 times greater than the three samples from the West Coast of Sweden. One herring from the sound between Sweden and Denmark had a much larger content of PCB (23 ppm on fat basis) than the herring from the rest of the Baltic area. This level increases the average value of this area from 4.6 to 6.8 ppm. This may suggest that there is a local contamination of PCB in the sound. There is an increase in content of DDT-compounds as well as PCB from the Gulf of Bothnia via the Stockholm Archipelago to the south of Baltic proper, indicating increasing sources of both these substances towards the south of the Baltic Sea.

The vagrant Baltic salmon has about ten times more chlorinated hydrocarbon residues than a sample reported from Great Britain (Holden and Marsden 1967).

The seals from the Baltic area generally contain large amounts of residues of both DDT and PCB, about ten times greater than the amounts found in Great Britain, Canada (Holden and Marsden 1967) and the Netherlands (Koeman and van Genderen 1966). Seal pups one week old from the Gulf of Finland also contained high levels of DDT-compounds and PCB and seal milk from the stomach from one of these pups had residues of the same magnitude.

DSW 202743

STLCOPCB4052907

The population of white-tailed eagle in Sweden seems to be on the decline. The number of probably fledged young per checked pair in Sweden has decreased from 0.80 in 1964 to 0.26 in 1969 and the number of successful breeding has decreased from more than 50 % in 1964 to about 20 % in the last few years (Helander 1970). All specimens of white-tailed eagle studied up to the present contain at least 3 accumulating substances known to be toxic. These are the DDT-compounds, PCB and mercury compounds (Berg et al. 1966). Figures for 4 birds are reported in table 3. Two specimens from northern Sweden, one of which had died by accident, contained of DDT-compounds 1.8 and 3.4 ppm respectively and of PCB 1.8 and 3.9 ppm in muscle tissue. The figures for specimens from the Archipelago of Stockholm are between 290-400 and 150-240 ppm for DDT and PCB respectively.

In fig 3 data are shown graphically for three substances (mercury based on fresh tissue and total DDT and PCB based on extractable fat) in three bird species from the Baltic, the black guillemot (Cepphus grylle), guillemot (Uria aalge) and cormorant (Phalacrocorax carbo sinensis). The means and range are indicated.

Guillemots from St Karlsö (site 9 in fig 3) almost invariably stay throughout their lives in the Baltic (unpublished data for ringed guillemots, Sten Österlöf). After breeding they move to the south part of the Baltic where they remain for the winter. In March and April they return to their breeding localities at St Karlsö.

In the guillemot eggs examined there is no difference in the content of mercury or in chlorinated hydrocarbon content between the years 1968 and 1969. Approximately the same levels of chlorinated hydrocarbons are found in fat from eggs and fat from the breast muscle of breeding guillemot. In three weeks old chicks the concentrations are somewhat lower. This may be due to a less contaminated food source in the breeding area and/or a slow accumulation process which gives young birds low concentrations in comparison with older ones.

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Black guillemots breed in the Archipelago of Stockholm (position indicated in figure 1) and spend the winter close to the edge of the ice. This is seldom situated south of Gotland. Although only four birds of this species were examined (two adults and two juveniles) it would appear that the figures for DDT-compounds and PCB in extractable fat are much lower in the juveniles than in juvenile guillemots. This difference is maintained in the adults for DDT-compounds but not for PCB where adults of both species have much the same values. This may indicate a lower contamination with DDT-compounds in the middle part of the Baltic as compared with the southernmost part. Conversely, the mercury figures suggest that adult black guillemots may have higher mercury content than guillemots. It is known that mercury contamination exists in the breeding locality of the black guillemot (Johnels et al. 1967).

Cormorants breed in a single locality close to the mainland west of Öland (site 10 in figure 1). They spend the winter in the Belgian area (Österlöf 1964, 1965, 1969). Although the juveniles examined had levels of chlorinated hydrocarbons in extractable fat only a little higher than those found in the black guillemot adult cormorants gave values rather similar to the high concentrations found in guillemots. The values for mercury are highly elevated in the old birds.

A large material of mainly various fish species is at the present time under study for elucidating the contamination with chlorinated hydrocarbons in the Swedish marine areas.

DSW 202745

STLCOPCB4052909

References

Berg, W., Johnels, A.G., Sjöstrand, B. and Westermark, T. 1966. Oikos 17 , 71.

Helander, B. 1970. Havsörnen i Sverige. Svenska Naturskyddsföreningen. With an English summary.

Holden, A.V. and Marsden, K. 1967. Nature 216 , 1274.

Jensen, S. 1966. New Sci. 32 , 612.

Jensen, S., Johnels, A.G., Olsson, M. and Otterlind, G. 1969. Nature 224, 5216.

Koeman, J.H. and van Genderen, H. 1966. J.Appl. Ecol. 2 ,99.

Koeman, J.H., Ten Noever de Brauw, M.C. and de Vox, R.H. 1969. Nature 221 , 1126.

Prestt, I., Jefferies, D.J. and Moore, N.W. 1970. Environ. Pollut. 1 , 3.

Risebrough, R.W., Rieche, P., Peakall, D.B., Herman, S.G. and Kirven, M.N. 1969. Nature 220 , 1098.

Risebrough, R.W. and Brodine, V. 1970. Environment 12 , 1.

Österlöf, S. 1964. Vår Fågelvärld. 273.

Österlöf, S. 1965. Vår Fågelvärld. 343.

Österlöf, S. 1969. Vår Fågelvärld Suppl. 5 , 17.

DSW 202746

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Fig 1. Position of localities and areas mentioned in the text.

Fig 2. Levels of PCB on extractable fat basis in pectoral muscle of starling (Sturnus vulgaris). Samples of adults and juveniles from 1967, 1968 and 1969. Dates of samples indicated. Positions of localities in fig 1.

Fig 3. Concentrations (means and ranges) of mercury and chlorinated hydrocarbons in birds from the Baltic.

STLCOPCB4052911

SAKAB

PCB - Possibilities of destruction

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Sweden

In March this year, SAKAB received an inquiry from AB Liljeholmens Kabelfabrik concerning the possibilities of destroying PCB-contaminated scrap windings. The total amount of scrap was given as 20 tons per year, containing about 7 tons of PCB. We were also told that liquid PCB wastes are returned to the manufacturer for reclaiming.

Chlorobiphenyls are very stable substances as are the parent molecules, benzene and biphenyl. Benzene is very resistant to strong alkalis, acids and oxidizing agents as boiling alkaline permanganate. Biphenyl is even more resistant to chemical action. On the other hand both are inflammable. Chlorination of benzene, biphenyl, as well as terphenyls, higher polyphenyls and naphtalenes give compounds which are both chemically very resistant and less flammable or non-flammable. Pentachlorobiphenyl, $C_{12}H_5Cl_5$, which contains hydrogen and chlorine in equivalent amounts, decomposes in an electric arc and gives a gas which contains almost pure hydrogen chloride. The explosion risk is therefore eliminated when using pentachlobiphenyl as a cooling and dielectric liquid in high-voltage electrical apparatus. Trichlorobenzene, $C_6H_3Cl_3$, has the same characteristics with respect to arc decomposition and is therefore often used in mixtures with pentachlorobiphenyl.

Because of the high chemical stability of PCB we were of the opinion that the most simple and economic way of destroying it would be by combustion. However, in Swedish periodicals, newspapers and elsewhere it has been stated that PCB cannot be destroyed by combustion[1, 2, 3]. We believe that this view is due to a misinterpretation of statements in the literature[4, 5, 6, 7]. There PCB is called "extremely stable", "inert", "non-flammable", "highly resistant to thermal degradation", "non-oxidizing", "chimiquement stable et inerte". Indeed, in one brochure pentachlorobiphenyl and higher PCBs are named "incombustible"[8].

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In order to settle the matter of whether PCB is combustible or not, we asked professor Nils Ingri, head of the department of inorganic chemistry at the University of Umeå to help us. In collaboration with docent Erik Rosén and fil. mag. Lars Karlsson he started a project on the thermal decomposition of PCB at high temperatures (1000 - 1500°K).

Naturally, they started with thermodynamic calculations of the systems. According to these calculations hexachlorobiphenyl, $C_{12}H_4Cl_6$, at 1200°K, decomposes to HCl, Cl_2 and C if there is no oxygen present. (Small amounts of CCl_4 and Cl were also found.) When adding oxygen to the system in amounts of less than six moles per mole of hexachlorobiphenyl CO is also formed. (As are small amounts of $COCl_2$.) If more than six moles but less than 12 moles of oxygen are added no C is formed but one gets CO_2 instead. When adding more than 12 moles of oxygen some H_2O is formed but most of the oxygen does not react. (Small amounts of HClO and ClO are also formed.) The necessary thermodynamic data for PCB compounds are not given in the literature but calculations showed that C_6H_5Cl and C_6H_6 should be present in concentrations of less than 10^{-8} mole per mole hexachlorobiphenyl, which is good evidence that the PCB molecules are not stable under these circumstances.

Recently, some simple experiments have been made at Umeå with tetra- and hexachlorobiphenyls. These substances were rapidly and completely destroyed when exposed to air at 900 - 950°C. In the gas streams from the reaction tube carbon dioxide and chloride were easily detected.

SAKAB also asked the manufacturers exporting PCB to Sweden - Prodelec, Bayer, Monsanto - about the possibilities of getting PCB reclaimed or destroyed by them. A short time ago we got the answers. According to these they are prepared to accept impure PCB of their own manufacture for reclaiming or destruction. The destruction will be made by combustion at a temperature of 800°C or higher. Monsanto indicated a cost for this service of about 350 kr per ton not including freight charges.

Recently, Monsanto also published an excellent paper discussing pollution control and waste disposal with respect to PCB[9]. According to this paper, in the case of liquid PCB, consideration should first be given to re-claiming. For waste disposal two methods are discussed, namely dry tipping and incineration. Incineration should be carried out at 800°C with

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a minimum residence time of 10 seconds. At 1000°C three seconds will be required. Open hearth incinerators are not considered suitable; their use would only serve to volatilise the PCB and pollute the atmosphere. Suitably designed tube incinerators are considered satisfactory. The effluent gases should, of course, be scrubbed and neutralized. For solid wastes another route is proposed. The first stage would consist of a rotary kiln furnace, operating at below 800°C, to volatilise the PCB. This would then pass to the second stage for combustion at 1200°C. It is recommended that accidental spillages of liquid PCB should be absorbed on dry sand, ash or sawdust and treated as solid waste. Dry tipping is considered to be a temporary measure which should be abandoned when facilities for proper incineration have been organised.

The incineration of liquid wastes, containing up to 70 % of chlorine, at Bayer, Leverkusen, has been described in two papers[10, 11]. The wastes are sprayed into a separate flame tube by means of atomizing burners operating on steam, and the combustion temperature is about 1200°C. An appropriately designed afterburner provides for a long residence time of the waste gases. Hydrochloric acid is formed, and may amount up to 30 g/Nm^3 in the unpurified gas. The hot waste gases are cleaned in a subsequent scrubber, being cooled down to temperatures below 70°C while saturated with water at the same time. Entrained dust particles in the gases are retained in a subsequent wet electric filter. The efficiency of the scrubbing unit is about 95 %.

Solid waste containing PCB does not necessarily have to be incinerated. It is quite possible to leach such wastes with a suitable solvent and deposit the decontaminated waste. The solution containing PCB may be incinerated or separated by destillation into PCB, which may be reclaimed, and the solvent used again. Tests at SAKAB indicate that acetone is a suitable solvent. However, leaching is probably more expensive than incineration.

To-day SAKAB has no facilities for destroying PCB but presumably within three years' time we will have an incinerator for chlorinated hydrocarbons installed at the new treatment plant to be built in the west of Sweden. However, it is already possible now to have liquid PCB returned to the PCB manufacturers for reclaiming or destruction.

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PCB Conference, Stockholm, Sept. 29, 1970
National Environment Protection Board

PCB - experiences in Finland

Matti Helminen, State Game Research Institute, Helsinki

The occurrence of PCB in the environment in Finland was verified in 1967 by the State Veterinary Medical Institute. Analyses of chlorinated pesticides were made, for instance, in connection with the international research project coordinated by OECD, and PCB was found in all samples or tissue homogenates of mussel, pike and starling representing brackish water, fresh water and terrestrial food chains, respectively.

Only the research team formed by Miss Eeva Karppanen, Mr. Kurt Henriksson and the author has so far been working with the occurrence of PCB in animal samples in Finland. Further analyses of PCB in animal samples of various species have confirmed the idea of common occurrence of small amounts of PCB in the environment in Finland. The concentrations found do not differ considerably from those found in Sweden and Norway. About 100 fish samples reveal that concentrations in samples from coastal waters are higher than in those from inland lakes. Pike samples from the delta area of the river Kokemäenjoki (Kumoälv) show signs of local contamination.

Highest concentrations have been found in ringed seals from the Gulf of Finland (mean of 8 speck samples 40.1 ppm in fat). The two white-tailed eagles from northern and eastern Finland had high contents but lower than those from the archipelago of Stockholm (Jensen et al., 1969, Nature 224: 247-250). Amounts found in 6 ospreys show considerable variation. In general, avian and mammalian predators belonging to aquatic food chains have had highest concentrations. Only six avian eggs have been analysed. Two addled eggs of eagle owl had 24.2 and 21.3 ppm and 4 osprey eggs 0.4 - 5.6 ppm in fresh sample.

According to inofficial commercial statistics available roughly 250 tons of PCB are used annually in Finland. The main consumers are manufacturers of electric equipment (more than 200 tons) and paint industry (30-40 tons). Additionally, many imported products of chemical industry contain PCB but the amounts are unknown.

In February 12, 1969 some parliament members made a proposal to the government emphasizing the hazards caused by the spread of PCB in the nature. This proposal did not lead to any action. In March 4, 1970, the Minister of Agriculture in his oral answer to the question made by two parliament members stated that the government considers research on PCB necessary. It should be known where and how much PCB is used in Finland and the possibilities of substituting PCB with less toxic chemicals should be explored. This task was given to the National Poisons Board formed by the Ministry of Social Welfare and Health in January 1970. The environmental section of the board is collecting information on the use of PCB in Finland at the present time. Lack of official sales statistics of products containing PCB is a great handicap also in Finland.

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PCB Conference, Stockholm, September 29, 1970.

PCB AS CONTAMINANT OF THE ENVIRONMENT-HISTORY

Lecture held at Wennergren Center [redacted]

Sören Jensen x)

Environment Protection Board SLL, Lantbrukshögskolan,

Uppsala 7, Sweden

x)
The work on the identification of PCB was done at the Institut of Analytical Chemistry at the University of Stockholm between 1964 and 1966.

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The history of the polychlorinated biphenyls (PCB) started in 1929 when industry introduced it for use as non inflamable oils in electrical transformers, condensers and in paint.

Up to 1966 no one had any idea that PCB was slowly entering nature, but to day we know that PCB after forty years use is almost as wide spread as DDT and can be found all over the world.

The reason for the accumulation in living organisms are close related to its chemical and fysical properties. Because of its non degradebility wastes will stay in the environment for a considerable time. Here it will enter the organisms stored in the depot fat and pass along the food chains in still increasing amounts.

Even before 1966 there were indications in litterature that PCB was present in wild life samples. As the chromatographic pattern was totaly unknown the authors just called them avian compound number 1, 2, 3 and so on if the sample came from birds. One author found the same pattern in air samples from London. After the PCB was first identified it was easy to confirm the findings.

It lasted untill November 1966 before it was shown that most unknown components from pesticide analysis from wild life samples were PCB. To day the PCB research is still increasing all over the world and one hundred laboratories are dealing with it in the United States only.

In November 1966 I had the opportunity to give a lecture in this building. The lecture was called PCB A NEW POLLUTANT, but it was never printed in english and as many present here to day have not heard it, I will give the head line again:

As the title on this lecture state, I will to-day tell about the discovery of some hitherto unobserved chlorinated hydrocarbons having up to eight chlorine atoms per molecule and found in residue analysis.

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is covered with a few mm of metylen chloride, this will be suckt up in the dry layer of silicagel and reach the upper end of the plate. Now the fat has a greater affinity to the powder on the plate than the chlorinated hydrocarbons has.

The fat front appears visible against a lamp, and with the aid of a razor blade the zone above the fat is transferred to the elution tube and the chlorinated pesticides adsorbed on the powder can now be eluted by one ml of ether. This solution has a concentration sufficient for detection of the chlorinated hydrocarbons down to the 10^{-12} g level.

The next step in the procedure is to separate the different chlorinated hydrocarbons from each other. It is easy to estimate what is not present but, more difficult to say exactly what is present. We suffer from the negative conclusiveness, as will be shown later.

The separation is accomplished by means of a gaschromatograph fitted to a detector that transfer its impulse to an recorder .

The system is shortly described:

A glas tubing with an inner diameter of 2 mm and about 2 m in length is filled with a support covered with a thin layer of an oil. The tubing is heated in the chromatograph to about 200°. Through the tubing a stream of nitrogen flow continously. When about 10 µl ($\frac{1}{100}$ part of a ml) of the purified biological extract is injected into the tubing, the components of the sample will be evaporized and go forward through the column with the gasstream. As the components have a different affinity to the collumnfilling they will pass through the column with different speed, and will reach the detector at the other end of the glasstubing at different times, the retentiontime, which will have a specific value for a certain compound. This is true, but unfortunately it is also true that two components may have the same retentiontime. This is one of the biggest problems in gaschromatographic analysis of unknown samples, as will soon be obvious.

To make it possible to estimate the retentiontime it is necessary to visiualize the presence of the chlorinated hydrocarbons. For the purpose more or less specific detectors are used.

The most common detector used in pesticide analysis is the electron capture detector. This can detect down to picogram level. Unfortunately this detector is not specific for chlorine, but gives also answer for oxygencontaining compounds. The response is here much lower, but can be counterbalanced if the concentration of the oxygen containing impurity is much higher.

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The system here described has, as we have seen two disadvantages:

1. Two different compounds can have the same retentiontime and be detected as one.
2. A registrated peak does not need to contain chlorine because the detector is not specific.

If the same sample is injected into two different columns with different chemical properties we have increased the possibility for a good separation in one of them.

When it seems doubtful wether the compound beeing responsible for a certain peak contain chlorine or not, it is possible to concentrate the sample and to analyze on a less sensitive but chlorine specific detector such as the microcoloumetric. As is seen from the two last pointed possibilities it is possible to reach a rather high degree of certainty in residue analysis.

When using the method just described we very often found that many chromatograms from residue analysis of most carefully purified biological samples still contained a large number of peaks, some of which had retentiontimes that did not agree with any known chlorinated pesticide or their metabolites. The chromatogram from an eagle can be an example. It was obtained by residue analysis of a sea-eagle found dead in the Archipelago of Stockholm. In the range of the known peaks, there were so many unidentified that there also must be an obvious risk that the known peaks as DDT could be covered by unknown.

If this remark is found true, the reported results of many previous quantitative analysis must be brought into question. Thus a chromatogram from analysis of human fat on a SF 96 column gave peaks with retentiontimes in agreement with the presence of DDE and DDT (op and pp). The next picture shows the same sample analysed on a QF 1 column. Now the two DDT-peaks have divided into four and two of them are still in agreement with DDT (op and pp). The two new peaks were unknown.

The first hypothesis was that the unknown peaks could come from compounds naturally belonging for instance to a pike. Against that spoke that neither treatment with alcoholic potassium hydroxide nor concentrated sulphuric acid changed the chromatogram in other ways than DDT disappeared and the DDE peak growed higher in the alkaline treatment, because of dehydrochalogenation of DDT. These treatments made it rather sure that the compounds did not contain oxygen or nitrogen. Furthermore the biologists collected pikes from the South to the North of Sweden. As the peaks decreased in the same direction it was rather obvious that they came from some kind of pollutants.

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It was also surprising that the peak height of the unknown compounds increased when going up through a food chain such as from young salmon via pike to sea eagle and that the late peaks increased relatively to the early ones in the same direction (see fig. 3).

Logically these unknown components were also thought to be unknown metabolites of the pesticides. Against this hypothesis spoke that eagle feathers collected at the Museum of Natural History from 1880 up to now showed the presence of the unknown peaks in the feathers in 1943 and the modern chlorinated pesticides were not introduced until 1945.

Residues of organic mercury have been investigated rather intensively in the Swedish fauna. As these compounds have good responses to the electron capture detector it was also investigated if the unknown peaks could originate from mercury compounds. It was shown that the water ecological system has high residues of both mercury and the unknown substances, when the same individuals were analysed. On the other hand pheasants suffering from mercury poisoning contained only low levels of electron capturing compounds, and these belonged to the normal insecticides.

The eagle sample giving the chromatogram shown in fig. 1 could be estimated to contain DDT and DDE up to 13 g/kg (13000 ppm) in the extractable fat. The amount of unknown compounds were suggested to be of the same magnitude and then sufficiently high to be analysed on the combined gaschromatograph-mass spectrometer. If this could be done succesfully it would be possible to get very important information about the chemical composition of the unknown components. For instance, the molecular weight and the numbers of chlorine atoms per molecule. This method gives the highest degree of certainty in the low level analytical chemistry. Amounts down to 100 ng of substance are sufficient.

Fig. 4 shows the mass spectra from the different unknown peaks in the eagle sample. The molecular weights could be estimated to 324, 358, 392, 426. Astonishingly the molecular differences were constantly 34 mass units. This difference for the first time shows a relationship in origin of the unknown components.

As chlorine exists as a mixture of two isotopes with atomic weights of 35 and 37 in the proportions of 75:25, a molecule with one chlorine atom will give two molecular peaks one for Cl^{35} and one Cl^{37}. If there are two chlorine atoms per molecule there are three possibilities: one molecule with Cl^{35}, one with both Cl^{35} and Cl^{37} and one with two Cl^{37}. Therefore there will

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be different intensity of the molecular peaks according to this. For x chlorine atoms in the molecule there will be molecular peaks for M^++2x. This relation can be read in tables and thus used for the estimation of the number of chlorine atoms per molecule:

Molecular weight	324	358	392	426
Chlorine content	5	6	7	8

An explanation of the close relationship between the different compounds can be given if one molecule is built up from the former by substituting a hydrogen with a chlorine, thus:

$$RH + Cl_2 \longrightarrow RCl + HCl$$
$$M \qquad\qquad M+34$$

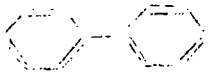
It is then possible to calculate the molecular weight of the parent hydrocarbon (PHC):

$$M_{PHC} = M - xM_{Cl} + xM_H$$

where M is the molecular weight of the component having x chlorine atoms. Doing this for M = 426 and 8 chlorine atoms per molecule (see above) we get the molecular weight of the parent hydrocarbon:

$$M_{PHC} = 426 - 280 + 8 = 154$$

The most probable formula for a substance giving this molecular weight and containing carbon and hydrogen is $C_{12}H_{10}$. This can only be satisfied when the parent hydrocarbon is biphenyl



and the unknown compounds must then be polychlorinated biphenyls (PCB).

This explanation was later fully verified by injection of a synthetic PCB on the mass spectrometer. Furthermore, extensive gaschromatographic investigations proved that the PCB standard gave peaks with the same retentionstimes as the unknown peaks from the wild life samples such as the sea eagle shown in Fig. 1.

In residue analysis are most often present 14 PCB-peaks. By means of mass-fragmentography 26 different are indicated. From a theoretical point of view 210 are possible containing from 1 to 8 chlorine per molecule. With even distributed chlorines on the two benzene rings and from 4-8 chlorine per molecule (the number most often found in residue analysis) there are still 104 different possibilities.

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Fig. 2

Chromatogram from extract of human fat on SF96 column.

Peak 9 and 10 overlapped DDT op' and pp' on the SF 96 column.

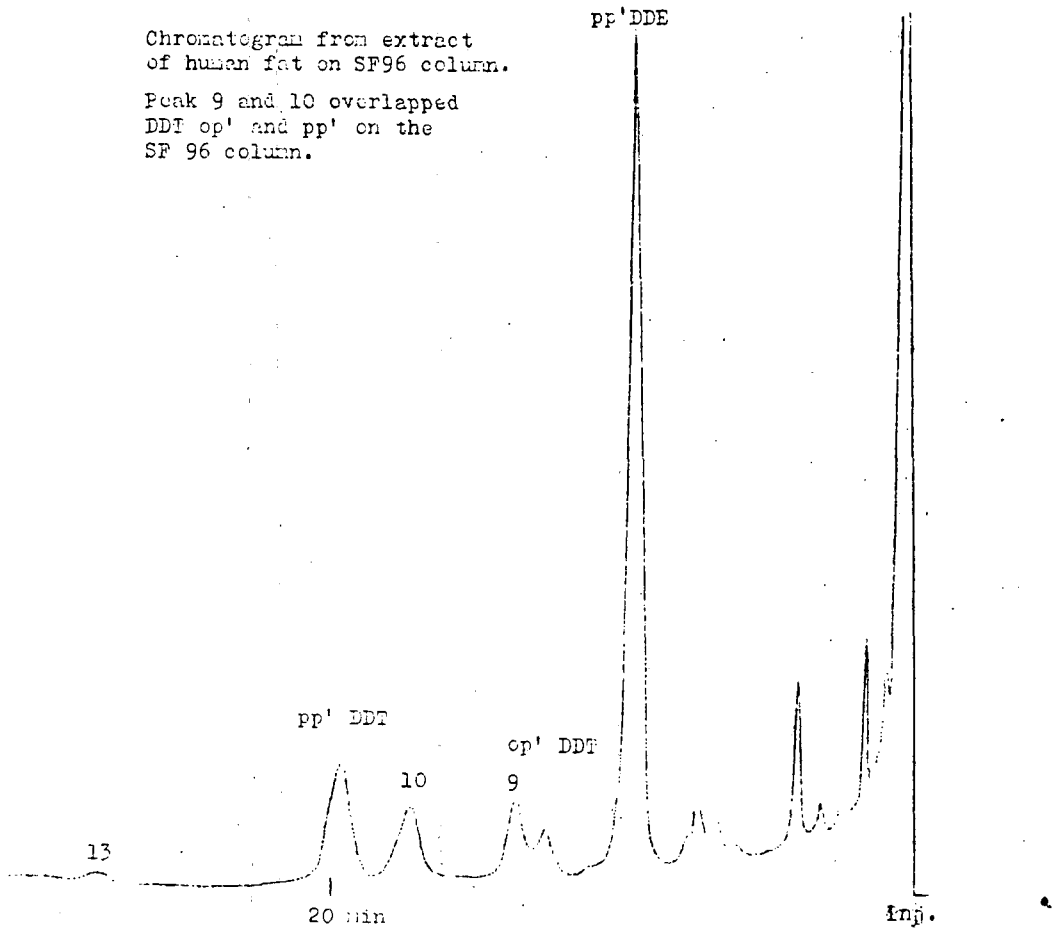

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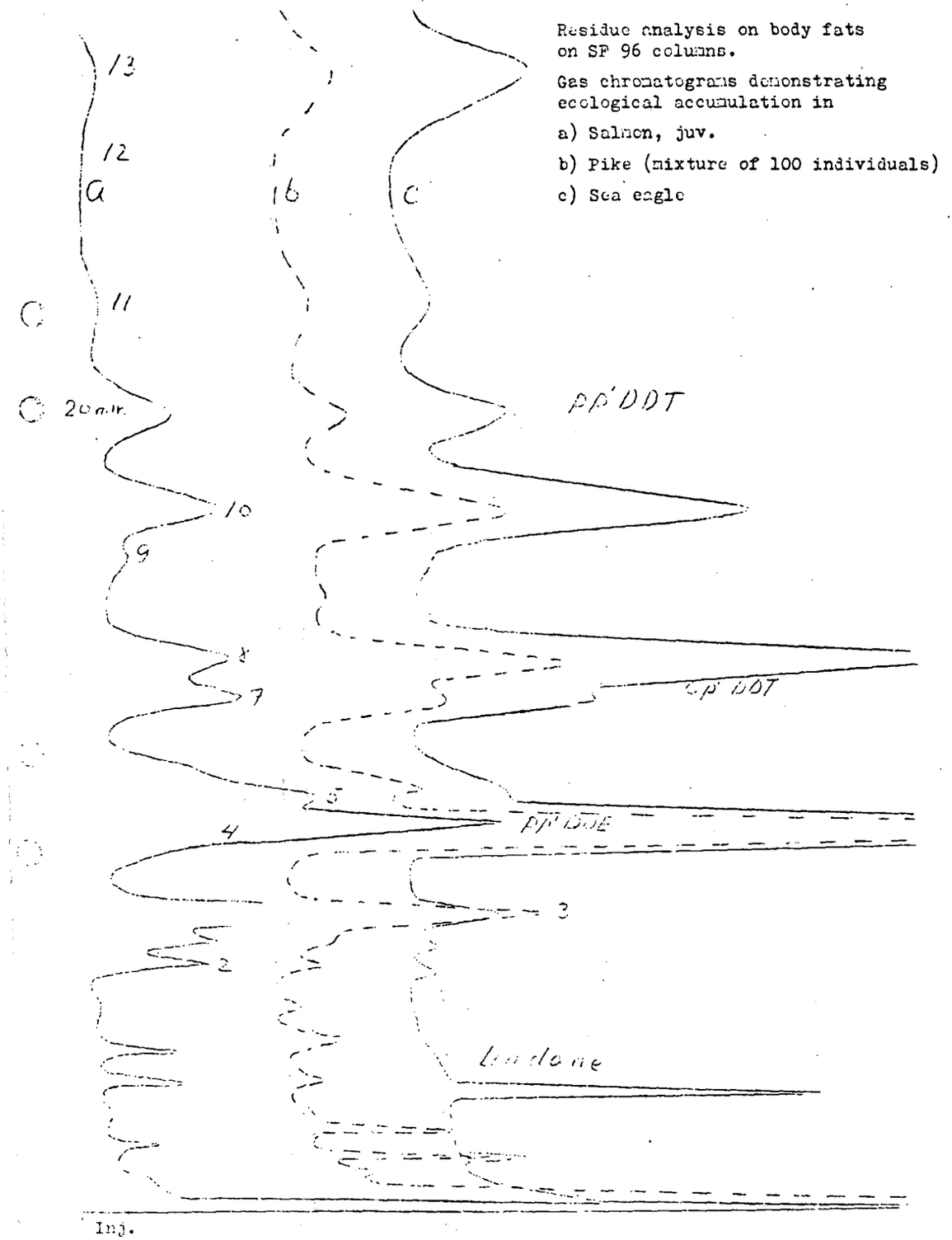
Fig. 3



Residue analysis on body fats on SF 96 columns.

Gas chromatograms demonstrating ecological accumulation in

a) Salmon, juv.

b) Pike (mixture of 100 individuals)

c) Sea eagle

Ing.

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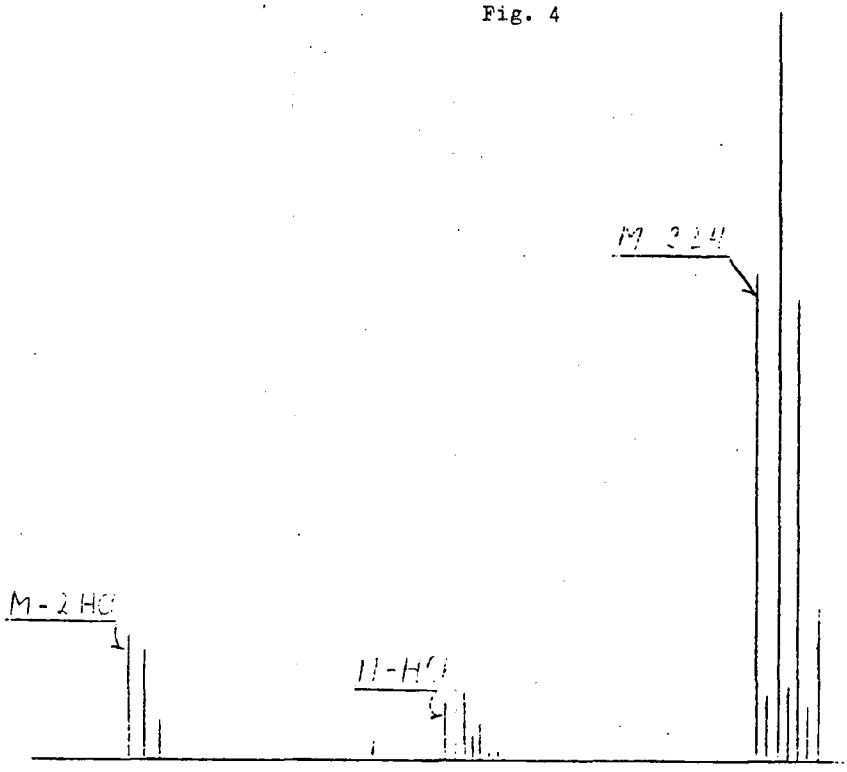
Fig. 4



Mass spectrogram corresponding to peak VII below

Chromatogram from combined mass spectrometry-gaschromatography of sea-eagle extract. Main molecular weights for I, II, III, IV and V: 282, 296, 310, 324 and 338 (saturated hydrocarbons), VI: 316 (DDE), VII, VIII, IX, XI and XII: 324, 358, 392, 392 and 426 (chlorinated biphenyls with 5, 6, 7, 7 and 8 chlorine atoms).

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NATIONAL ENVIRONMENT PROTECTION BOARD
Research Secretariat
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September 9, 1970

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P R O G R A M

for

P C B Conference

September 29, 1970

in the "Werner Gren Center" (session hall, annex building)
Arrenvägen 166, STOCKHOLM

Chairman: [illegible] FRIBERG, Prof., M.D., Natl. Inst. of Public Health, STOCKHOLM

0900-0910 Introduction
[illegible], Ph.D., Natl. Environment Protection Board, SOLNA

0910-0930 PCB as contaminant of the environment - history
[illegible], Ph.D., Natl. Environment Protection Board, SOLNA

0930-0940 Discussion

0940-0955 PCB - uses in industry
[illegible], Ph.D., P C U D E L F C, LYCKES (France)

0955-1015 PCB - experiences in Finland
[illegible], Ph.D., State Game Research Inst., HELSINKI (Finland)

1015-1025 Discussion

C o f f e e b r e a k

1045-1105 PCB - occurrence in Swedish wildlife
[illegible], Prof., Ph.D., Sw. Museum of Natural History, STOCKHOLM

1105-1115 PCB - effects on mammals
[illegible], Prof., M.D., Univ. of Lund, LUND

1115-1125 PCB - effects on birds
[illegible], D.V.M., Natl. Poisons and Pesticides Board, STOCKHOLM

1125-1135 PCB - indications of effects on fishes
NILS JOHANSSON, Ph.D., Salmon Research Inst., ÄLVKARLEBY

1135-1145 Discussion

L u n c h

1300-1310 PCB - synthesis and radioactive labelling (≦ 4 Cl-atoms)
[illegible], M.Sc., Univ. of Lund, LUND

1310-1320 PCB - synthesis and radioactive labelling (≧ 4 Cl-atoms)
CARL AXEL MAGNUSSON, Ph.D., Univ. of Stockholm, STOCKHOLM

1320-1330 PCB - possibilities of destruction
[illegible], D.Eng., A A K A B, STOCKHOLM

1330- G e n e r a l d i s c u s s i o n

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