Chlorobiphenyls (PCBs) in Wisconsin Natural Waters

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ABSTRACT

The seizure of fish from Lake Michigan because of the presence of chlorinated pesticides and PCBs at concentrations which are greater than that regarded as safe for human consumption has demonstrated that the continuous input of trace quantities of these chemicals may jeopardize major food resources for man. Recent evidence has indicated that the ratio of the PCB concentration in fish to that in the water near the site of capture is on the order of 100,000, although the ratio may vary by more than an order of magnitude. The magnitude of this ratio suggests that the concentration of PCBs in fish from water containing greater than 0.05 µg/l PCBs may exceed the 5 µg/gm action limit established for fish.

Investigations by the Water Chemistry Program and the Wisconsin Department of Natural Resources have shown the PCBs are present in the wastes from all municipalities examined. The concentration of PCBs in the receiving waters for sanitary wastes alone seldom exceeded 0.10 µg/l, whereas the discharge of industrial wastes containing PCBs either directly or in municipal waste effluents resulted in concentrations greater than 2 µg/l (as Aroclor 1248).

Efforts to determine the major sources and current levels of PCBs in the Green Bay area of Lake Michigan have shown that the concentrations of PCBs varied from below the determinable limit of approximately 0.01 µg/l (20 liter water sample) in rivers such as the Big Suamico which drain agricultural and forested areas and receive no municipal wastes to 0.45 µg/l (as Aroclor 1254) in the receiving streams for industrial and municipal wastes. The mixtures of PCBs in municipal wastes generally resembled Aroclor 1248 or 1258 although the earlier eluting isomers of Aroclor 1242 were often observed. The concentration of PCB mixtures resembling Aroclor 1254 in the Pestigo and Oconto Rivers were 0.31 and 0.45 µg/l, respectively, in November, 1970; 0.38 and 0.16 µg/l, respectively, in April, 1971; but decreased to near or below the detectable limit during the summer of 1971. The concentration in lower Green Bay water varied from 0.04 µg/l at Sturgeon Bay to 0.07 µg/l near Longtail Point. Further study is required to determine if the restricted use of PCBs in industry will lead to a pronounced decrease of the concentration of the chemicals in waste effluents and in nearshore environments such as Green Bay.

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Polychlorinated Biphenyl Residues in the Milk of Environmentally and Experimentally Contaminated Cows

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One of the PCB's, Aroclor 1254, has been used in coatings for silos. Milk samples obtained from the six farms with these silos contained PCB residues in the range of 5 to 11 µg/g milk fat when silage was fed. When silage was not fed, detectable levels in the range of 1 to 2 µg/g milk fat were present.

On one farm the cows had been simultaneously exposed to PCB and high levels of DDE. The cows were removed from the sources of both contaminates at about the same time. Milk samples were obtained from the individual cows at 3 week intervals for 6 months. Concentration of the PCB in milk declined about 50% within 3 weeks after removal from silage. Subsequently, the rate of decline was 1.3% per day. The rate of decline of DDE concentration in milk fat was also 1.3%. The correlation between rates of the two compounds for individual cows was 0.82.

In a laboratory experiment, 9 cows with varying stages of lactation and levels of production were fed 200 mg per day Aroclor 1254 for 60 days. Milk samples were obtained during the 60 day feeding period and the following 60 days. Level of residue in milk approached but did not reach equilibrium at 60 days. The concentration at 60 days was 50 µg/g in the milk fat. The total quantity excreted in milk was 35 µg per day. When the PCB feeding stopped there was an initial decline to 24 µg/g within 10 days. Thereafter the concentration decline was approximately 1.0%/day. Concentration of PCB in the milk fat was not related to stage of lactation or level of milk production.

In both the environmental and laboratory work only the more highly chlorinated fractions of the Aroclor 1254 were excreted into milk. For these fractions the residues appeared to behave identically to DDE in all parameters that were studied.

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Contamination of the Bay of Fundy - Gulf of Maine area with polychlorinated biphenyls, polychlorinated terphenyls, chlorinated dibenzodioxins, and dibenzofurans.

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Modified cleanup-chromatography of Holden and Marsden was used to determine PCB and polychlorinated terphenyls (PCT) in aquatic animals. GLC on 3% OV-210 on Chromosorb WAW 60/80 in a 6 ft x 4 mm glass column at 200°C was used to detect PCT. Chlorinated dibenzodioxins and dibenzofurans were determined after additional chromatography on alumina essentially according to Porter and Burke. All samples contained PCB in most cases closely resembling Aroclor 1254. Higher chlorinated biphenyls of the Aroclor 1260 variety were also present in some samples, particularly in eggs and tissues of aquatic birds. PCT were detected only in eggs and tissues of herring gulls in levels of 0.1 and 1.4 μg/g wet weight as Aroclor 5460, respectively. Chlorinated dioxins and

dibenzofurans were not detectable (detection limit 0.01-0.04 μg/g wet weight, depending on the degree of chlorination) in any of the examined samples: muscle and liver of white shark, eggs of double-crested cormorants and herring gulls, commercial herring oil, and groundfish-herring fishmeal. The levels of PCB and chlorinated hydrocarbon pesticides are summarized below:

Species	Tissue	μg/g wet weight PCB (as Aroclor 1254)	p,p'-DDE	Other
Herring *Clupea harengus*	whole fish	0.34	0.06	
Mackerel *Scomber scombrus*	muscle	0.35	0.07	
Plaice *Hippoglossoides platessoides*	muscle	0.38	0.01	
White hake *Urophycis tenuis*	muscle	0.44	0.03	
Ocean perch *Sebastes marinus*	muscle	0.32	0.03	
Cod *Gadus morhua*	muscle	0.55	0.04	
Sea raven *Hemitripterus americanus*	muscle	0.21	0.08	
	viscera	0.73	0.30	DDT 0.24
Basking shark *Cetorhinus maximus*	muscle	0.07	-	
	liver	1.07		
White shark *Carcharodon carcharias*	muscle	0.77	0.48	
	liver	218	335	DDT 63 DDD 43
Bluefin tuna *Thunnus thynnus*	muscle	1.54	0.15	
Herring oil		3.55	2.27	DDT 0.37
Fishmeal		0.54	0.19	

Species	Tissue	PCB (as Aroclor 1254) µg/g wet weight	p,p'-DDE µg/g wet weight
Double-crested cormorant *Phalacrocorax auritus*	eggs	43.5*	29.4*
		17.2*	8.6*
	muscle	3.38	8.40
	liver	2.13	4.16
	Subcut. fat	38	164
	Abdom. fat	52	162
Herring gull *Larus argentatus*	eggs	12.6*	5.67*
		5.54*	2.83*
	muscle	5.06	2.07
	liver	6.50	2.08
	Subcut. fat	75	26

*Different nesting colonies

ABSTRACT

ACCUMULATION OF POLYCHLORINATED BIPHENYLS IN ECOSYSTEMS

Insufficient data on production and use figures of the polychlorinated biphenyls in industrialized and developing countries have hindered efforts to construct mass balance equations for major sinks such as the oceans. Determination of the long-term accumulation trends in these sinks, essential for the prediction of future effects upon fisheries and wildlife, would require accurate estimates of potential input. Considerable evidence suggests that aerial dispersal contributes PCB to marine ecosystems, but large amounts also enter the sea as components of industrial wastes. The relative importance of each pathway on a global scale has not yet been determined. Arctic ecosystems remote from input sources are contaminated with PCB and in the southern hemisphere marine birds carry substantial body burdens. South of the Antarctic Convergence, however, PCB residues in resident wildlife are considerably lower and in some samples are not detectable.

Comparatively high residues in Atlantic zooplankton suggest the need for studies of the interrelationships between trophic accumulation in aquatic food webs and partition coefficient phenomena based upon physicochemical equilibria between very low concentrations in ambient water and higher concentrations in lipids of organisms.

Populations of birds with exceptionally high PCB residues include the remaining Peregrine Falcons in North America and the Ospreys of the north-eastern United States.

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Abstract

Photochemical Behaviour of Chlorobiphenyls (PCB)

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It has been shown previously (1) that 2,2',4,4',6,6'-hexachlorobiphenyl, when irradiated at 3100 Å in hexane and methanol solutions, photolyses rather readily to give products which are formed by stepwise loss of chlorine, rearrangement and condensation. "Polar" products whose structures were not further investigated were also observed.

We have now begun a systematic study of the photochemical behaviour of chlorobiphenyls with emphasis on two areas.

(i) Relationship between the structure of chlorobiphenyl isomers and their photochemistry. A series of isomers including compounds which are major components of commercial PCB preparations are being used.

(ii) Influence of irradiation conditions on the nature and amounts of products formed. In this study pure chlorobiphenyl isomers as well as commercial PCB preparations are being used and the effect of natural sunlight and a number of artificial light sources is being investigated. The irradiations are carried out in the vapour phase, in aqueous suspension, hexane methanol and aqueous dioxane solutions and with thin films of undiluted material.

Relatively large amounts of dechlorinated compounds were among the decomposition products in all experiments. With pure

isomers, newly formed chlorobiphenyls were separated and characterized by TLC and GLC/Mass Spec. techniques. Changes in the commercial PCB preparations, on the other hand, were observed mainly by their altered GLC patterns.

Significant amounts of polar materials were formed when air and water were present during the irradiations. Chromatographically, some of these polar compounds behave similar to hydroxychlorobiphenyls whereas others may be carboxylic acids and polymers. The structures of these compounds which are now under investigation will be discussed as will be conditions for their formation.

The fact that polar products are formed from chlorobiphenyls under the influence of UV radiation could be important from several points of view. Hydroxychlorobiphenyls and related polar materials may no longer show the toxicity that is associated with chloroaromatic hydrocarbons. These compounds may also be more readily degraded by microorganisms in the environment. Hydroxychlorobiphenyls on the other hand may also be important as intermediates in the environmental formation of toxic chlorodibenzofuranes. Experiments designed to evaluate this possibility are underway and will be described.

(1) S. Safe and O. Hutzinger. Nature, 232, 641 (1971).

USE AND REPLACEABILITY OF POLYCHLORINATED BIPHENYLS

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SUMMARY OF REPORT

This report summarizes a collation of technical information on the uses of polychlorinated biphenyl compounds (PCB's), their advantages and disadvantages for various applications and the possibility of replacing them with alternative substances. Applications have been divided into four categories:

1. Dielectric fluids for capacitors and transformers.
2. Industrial fluids for hydraulic, gas turbine and vacuum pump uses.
3. Heat transfer fluids.
4. Plasticizers and miscellaneous uses.

The information was gathered from published literature, special reports, patent claims, and personal conversations with officials and scientific representatives of industrial and utility companies. Such trade information as was used has come from sources other than the manufacturer. The report is not exhaustive and it is likely that particular applications described do not currently involve the use of PCB's.

NONS 201047

The major value of the PCB liquids is that those with four or more substituted chlorines per molecule are non-flammable as are their decomposition products. Thus they can be used as fluids at temperatures up to 700°F without the danger of explosions and fire. The major disadvantage of the PCB's is their possible toxicity danger. The other comparable class of non-flammable fluids are the fluorocarbons which typically have a lower vapor pressure and lower boiling point than the chlorinated compounds.

PCB's are used in fluids (known as askarels) for electrically insulating and cooling transformers when the transformers are used in or near buildings. Mineral oils are the preferred fluids when fire does not create a hazard. Dry transformers can also be used but are larger and more expensive. Fluorocarbon liquids require a special transformer design. Fluorescent light ballast capacitors and phase correction capacitors utilize the high dielectric constant of the PCB's to effect significant reduction in capacitor size and cost. Few suitable fluids have a comparably high dielectric constant. Flammable fluids are not allowed by insurance companies and building codes in capacitors used in buildings. Replacement of PCB's in capacitors and transformers would require considerable time and money for reengineering, manufacture and application of substitute equipment.

PCB's are useful in hydraulic systems where leakage onto hot metal surfaces could cause a dangerous fire (e. g. aircraft hydraulic systems). Hydraulic fluids are also made with phosphate esters which are toxic and which will burn at high temperatures. Replacement of PCB's in some hydraulic systems could increase loss of life due to fire. Gas turbines require lubrication at high temperatures. PCB's can be used but tend to be corrosive. Phosphate ester lubricants seem better in this respect. Chemical stability is more important for high temperature lubricants than is non-flammability. PCB fluids are useful in diffusion booster

pumps to produce moderately high vacuums with relatively poor fore vacuums. Non-flammability is not especially important for diffusion pump liquids, and alternative liquids are available.

Flammable heat transfer fluids present a fire hazard if they leak into a furnace or onto hot aurfaces. The use of PCB's prevents this danger. In some cases water is a suitable substitute at moderately high temperatures. Other heat transfer fluids are commercially available and in use. Replacement of PCB'a ia aatisfactory in some, but may be dangerous in other heat transfer uses.

The PCB'a are good plasticizers for use with adhesives, textiles, surface coatings, sealants and copy paper. In some cases the PCB's act as fire retardants. There are no particularly unique properties of PCB's for plasticizer uses and equally effective alternatives are generally available (e. g. phosphate esters are often used as fire retardants).

Polychlorinated Biphenyls in Non-carbon Copying Paper

by

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In an effort to clarify the distribution of polychlorinated biphenyls (PCB) in our environment, we were suggested that PCB or its related compounds might be used for production of non-carbon copying paper. Three of 4 Japanese brands of the paper on the market were therefore collected and examined for PCB. The paper consists of three different sheets, namely upper, middle, and lower ones. These sheets of paper were separately extracted with acetone in a Soxleht apparatus for 3 days. The extracts were examined by a gaschromatography with an electron capture detector and 3 different liquid phases such as 5 % SE-30, 5 % QF-1, and 2.5 % DEGS coated on Chromosorb W AW DMCS. The gaschromatographic patterns observed were all identical with that of Kanechlor-300 (a brand of PCB) containing 43 % chlorine. The infrared absorption spectra and mass-spectra of the extracts also demonstrated the presence of Kanechlor-300 in them. The approximate concentration of PCB was 2-6 % in the upper and middle sheets and 0.02 % in the lower sheets.

To estimate how much PCB sticks to fingers by handling the copying papers, 3 independent sets of the upper, middle, and lower sheets, each consisting of 96 precut sheets of the same size, were counted by turning over all the sheets one by one by fingers. After counting, the fingers were washed with n-hexane once and the resulting n-hexane washings were examined for PCB. The amount of PCB in the washings, calculated as Kanechlor-300, ranged from 11.40 to 51.68 μg in 5 subjects.

It was proved thus that PCB in the paper easily sticks to fingers when we handle it. It was also demonstrated that only one third of the PCB sticking to fingers can be removed by ordinary hand washing with soap and water.

In addition to the above domestic brands of paper, a few other samples of non-carbon copying paper were analyzed for PCB. One of them was a brand made by one of the leading paper makers in Britain and was obtained as packed in its original container in May 1971. Analysis showed that both upper and middle sheets of this make contained PCB. The second sample which was of American make and obtained as packed in its original container in June 1971 also contained a considerable amount of PCB. Another sample of paper which was sent to us for examination by a company in Thailand, however, did not contain PCB. We were told that it is also of American make.

Nevertheless to say, the free use of PCB for this kind of very popular copying paper necessarily leads to the uncontrollable heavy direct exposure of general population to it. In view of its chemical and pathological characteristics, we consider that the use of PCB for the paper should be discontinued in any country in order to prevent another occurrence of "Yusho". The Japanese makers of the paper are said to have quickly acted along this line in April 1971. To our regret, however, there have been no control measures set up by the government against the use of PCB and a considerable amount of

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the PCB-polluted papers are still widely used in Japan.

RATES AND ROUTES OF TRANSPORT OF PCB's IN THE ENVIRONMENT

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ABSTRACT

The fragmentary information on routes, rates of entry, and ultimate fate of PCB's in the environment is reviewed with special reference to North America and adjacent parts of the oceans. Until very recently, only speculative information was available on production statistics and on quantitative patterns of use. Monsanto Chemical Company has now released figures for total U. S. sales of PCB's, which peaked at 36,500 tons in 1970, and has agreed to furnish supplementary information in time for the Durham meeting. PCB's have been used primarily as dielectric, heat transfer, and hydraulic fluids, particularly where fire resistance is important, and as plasticizers. Minor uses, accounting for less than six percent of sales, have included surface coatings and sealants, adhesives, printing inks, pesticide extenders, microencapsulation of dyes, and wax modifiers. Monsanto has recently restricted sales of certain products based on considerations of either the possibility of contamination of food products or the inability to monitor or control possible losses in the environment. Sale of PCB's for use as plasticizers and the minor uses presented above was discontinued in 1970. In addition, sale of heat transfer fluid has been curtailed and, for those applications which might lead to food contamination, discontinued. As a consequence of these measures, U. S. sales of PCB's by Monsanto have fallen by almost 50 percent to an estimated 18,800 tons in 1971. Concurrently with the control of sales Monsanto is reducing the average chlorine content of PCB's and expects that the percentage of PCB's containing 5 to 9 chlorine atoms per molecule will decrease from 44 in 1969 to 21 in 1972.

As in the case of most industrial chemicals loss figures for PCB's are practically non-existent. Some rough estimates of losses to the environment have been made from the nature of application, and the physical properties of the PCB's. These estimates suggest that a large fraction of the PCB's manufactured to date are confined in sealed containers, either in active service or discarded in dumps. Major routes into the environment appear to be to waterways and estuaries, through scrappage or leakage. Losses to the atmosphere by incineration, vaporization or co-distillation are probably relatively minor. No major direct routes to the terrestrial environment have been identified, since the past use of PCB's for pesticide formulations was small.

Additional evidence on the amounts and routes of PCB's in the environment is provided by data on environmental occurrence of PCB's, derived from both sparse measurements of PCB's in water and air, and more extensive measurements on PCB residues and ΣDDT/PCB ratios encountered in the biota. The spatial distribution of both PCB levels and ΣDDT/PCB ratios are consistent with the postulate that the discharge of PCB's into the environment has been concentrated near industrial areas, and that their subsequent transport has

been mainly through the movement of surface waters or water-borne particulates. The occurrence of PCB's in remote non-industrial sites suggests that a finite quantity of PCB's have also been transported by air-borne particulates. Co-distillation or vaporization is tentatively discounted as a major route, on the basis of the apparent lack of fractionation during transport in the environment amongst the refractory isomers of the commercial product.

PCB's have been identified in snow, rainwater, and air-borne particulates over the continental U. S., but could not be detected in particulates carried from the Pacific over the California coast. Roughly one thousand tons per year of PCB's is estimated as an upper limit for the annual deposits by air-borne particulates on the continental U. S. Estimates of the total input to the terrestrial environment, including contribution by irrigation waters, are two orders of magnitude lower than those for DDT. However, observed ΣDDT/PCB ratios in terrestrial animals are generally lower than 10. The discrepancy is so far unexplained.

The amounts of PCB's stored in freshwater fish and discharged in surface waters are relatively small. The total PCB content of freshwater biota in North America is estimated to be of the order of a few tons and the amounts discharged with surface waters, estimated from data on ΣDDT/PCB ratios in the biota, of the order of 100 tons.

The estimate of total input of PCB's into the marine environment off the U. S. coast from rivers and sewage sludge is of the order of several hundred tons per year, slightly greater than the estimates for DDT. Direct introduction to coastal waters, from industrial leaks, dumping of industrial wastes and dredge spoils, is of unknown magnitude, but probably accounts for locally high levels of PCB's and low ΣDDT/PCB ratios in coastal animals, especially on the Atlantic coast. Farther offshore, ΣDDT/PCB ratios are lower, consistent with the postulate that widespread aerial fallout is a more important route for DDT than for PCB's.

The major conclusion of the review is that only a small fraction of the total PCB production has entered the biosphere. Of the remainder, part has been destroyed by incineration, part is in captive use in heat exchanges or transformers, and part has accumulated in dumps or sediments in shallow waters from which it is leached out at an unknown but probably slow rate. The figures presented in the review are based on fragmentary evidence and are speculative; they emphasize the need for improved transport models for pollutants in the environment.

PCB and the Paper Industry - A Progress Report

The discovery of PCB-contamination of its products by the paper industry was described at a recent workshop sponsored by the Grocery Manufacturers of America, Inc. Research by the paperboard manufacturers first encountering the problem showed the primary souce of PCB to be carbonless carbon paper which was present in certain grades of paperstock being recycled in the paper i dustry. Polychlorinated biphenyl was being used as a component of the dye carrier in the carbonless carbon paper. It was discovered subsequently that PCB also could be present in papers and paperboards that did not contain carbonless carbon paper. The latter contamination is felt to reflect backgrounds levels of environmental PCB contamination.

The paper industry took immediate steps to eliminate carbonless carbon paper materials from all grades of paperboard used in the packaging of food. Simultaneously, a chlorine-free, biodegradable PCB substitute was developed for carbonless carbon paper use. No PCB has been used in carbonless carbon paper manufacture since June 1, 1971. In addition, Monsanto Company, Inc., sole United States manufacturer of polychlorinated biphenyls, discontinued sales of the material for other than closed syatem applications on September 1, 1970. Environmental levels of PCB must therefore be regarded as decreasing.

On behalf of the paper industry, the American Paper Institute is sponsoring a research study to:

1. Determine present levels of PCB in United States pulp, paper and paperboard

2. Investigate the migration of PCB from paperboards to foods

The migration study has not progressed sufficiently far for meaningful data to be available at this time. Preliminary data from the survey study, however, indicate 94.5 percent of the samples analyzed to have PCB contents less than 10 parts per million. Data from paper industry members also show substantial reductions to have been made in the PCB contents of recycled fiber paperboards during 1971.

These facts support the paper industry contention that PCB levels in currently-manufactured paper and paperboard food packaging materials do not represent a hazard to public health.

Paul Trout

Levels of polychlorinated biphenyls (PCB) in Swedish foods

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PCB has been used in industry in Sweden since 1929, from the beginning only in electric transformers and capacitors, later also in paints, lacquers etc. The importation of PCBs to Sweden in 1970 was close to 500 tons. Approximately half of this was exported to other countries in electric capacitors. The use of PCB in dyes was 65 tons in 1968, but was voluntarily diminished by industry to 35 tons in 1970.

The high degree of persistence and lipid solubility of the PCBs have led to their accumulation in many foods of animal origin. In Sweden they now occur in the natural environment roughly in the same amounts as the chlorinated pesticides (Jensen et al. 1969). The special hydrographical conditions of the Baltic favour the accumulation of PCB in certain species and areas. The water exchange with the North Sea is very limited because of the thresholds (8 m and 18 m) and the narrowness of the Danish Sounds. The wide archipelagos at some places will also make possible high concentrations in some localities with high pollution, e.g. outside of Stockholm.

PCB and organochlorine pesticides have been analyzed in food samples from 1967 and later in Sweden. After extraction and routine clean-up, the extract is separated by thin-layer chromatography into two groups, one of which contains PCB and p,p′-DDE, o,p′-DDT, o,p′-DDE. The PCB is determined by gas chromatography of this group before and after oxidation of the organochlorine pesticides. Some of the PCBs can be analyzed by gas chromatography without decomposition of the organochlorine compounds. Recoveries average 96-101 % for PCBs added to purified fish extracts before application to thin-layer plate (Westöö & Norén 1970 a).

There was less than 0.1 mg PCB/kg of the fat in all samples of butter (n = 154), margarine (n = 115) and various vegetable oils (corn, olive, soybean, sunflower, linseed, safflower, sesam and mixed oils, total n = 197) (Westöö, Norén & Andersson 1970).

There was less than 0.1 mg PCB/kg in such food items as chicken (n = 143), lamb meat (n = 26), lamb fat (n = 10), beef (n = 34), whole eggs (2 samples) and egg white (28 samples). Two samples of egg yolk (out of 185) contained PCB in the 0.2-0.5 mg/kg range; the same was true for 1 out of 107 samples of pork.

Cow's milk (n = 11) had less than 0.5 mg PCB/kg fat in all samples. Human milk had definitely higher levels of both PCB and "total DDT" than cow's milk. Thus in human milk (n = 22) p,p′-DDT averaged 1.24 ± 0.072 mg/kg fat, p,p′-DDE 2.08 ± 0.13 mg/kg fat and PCB 0.50 ± 0.039 mg/kg fat. With an average fat content of 3.2 % in the human milk, the PCB in whole milk averaged 16 ± 2.5 µg/kg (mean ± standard error).

Recently Westoo et al. (1971) also discovered PCB in some samples of cereal products. The origin was found to be the packaging material. On thin-layer chromatography and on gas chromatography the PCB behaved as PCB of type Clophen A 60. In several samples of one kind of imported children's food the PCB levels exceeded 1 mg/kg. In one sample of this product, which had been stored at room temperature for two years, the level of PCB had increased to 11 mg/kg. The sale of this children's food in Sweden was stopped in July 1971.

Westöö & Norén (1970 b) also analyzed for PCB and organochlorine pesticides in about 1 000 fishes from lakes, rivers, the Baltic Sea and the Atlantic Ocean. The levels depend on the fat content of the fish and on the geographical location. PCB levels higher than 0.5 mg/kg fish flesh were rarely seen in Sweden in lean fish (haddock, Gadus aeglefinus, cod, Gadus morrhua, plaice, Pleuronectes platessa) all of which have less than 2 % fat in the edible flesh.

In 54 samples there was more than 0.5 mg PCB/kg flesh, e.g. in salmon, Salmo salar (10-15 % fat), eel, Anguilla anguilla (25 % fat) and herring (7-15 % fat). The highest PCB levels were found in 2 small samples of herring from the Stockholm archipelago, 2.8 and 2.5 mg/kg, in 4 yellow eels close to Gothenburg, 1.5 mg/kg, and in a char, Salmo salvelinus, in the Vattern lake. - Cod liver is rich in fat (40-70 %). Livers of cod from the Baltic Sea had high levels of PCB, 3.8 mg/kg (2.4-4.9), and of DDT+DDE+DDD, 18 mg/kg (11-22). Because of the high levels of "total DDT", cod livers from the Baltic were declared unfit for human consumption (1971). A few livers of cod from the Swedish West coast also had high PCB levels, 2.5 ppm, but lower levels of DDT+DDE+DDD, 3 mg/kg, than in the Baltic.

Studies by Olsson et al. (1971) show that DDT levels in fish are fairly even over wide areas, whereas PCB levels decrease with the distance from the sources of pollution in the same manner as the methylmercury levels in fish.

The main intake of PCB via the food in Sweden thus occure by eating fish. The average consumption of fish in Sweden 1961-1969 was 17 kg/person/year - and of crustaceans and molluscs about 1 kg/year. In terms of fish flesh, Westin (1970) calculated an intake for 1965 of 35 g per person and day. Of this, about 2 g represent freshwater fish, 1 g caviar, and 1 g shellfish and molluscs together. The average intake of PCB therefore probably does not exceed 1 µg per day, but may exceed 10 µg/dag in people eating herring from the Baltic 3 times a week.

A suckling baby ingests about 0.15 litre of mother's milk per kg body weight daily in the 2nd and 3rd months of live, with an average PCB level of 16 µg/kg milk. This corresponds to 2.4 µg/kg body weight per day. It is known from Japan that PCB passes the placental barrier (Tsukamoto et al. 1969). Thus we have a situation similar to that with DDT with the suckling baby getting the highest exposure. This will probably be the case with several environmental pollutants with high lipid solubility.

To prevent further pollution with PCB the Swedish riksdag (parliament) has enacted the 1971 PCB Act, authorizing the government to prescribe conditions regarding importation, production, offering for sale or handling of PCB.

References:

JENSEN, S., A.G. JOHNELS, M. OLSSON, G. OTTERLIND (1969). DDT and PCB in marine animals from Swedish waters. Nature 224:247-250.

OLSSON, M., S. JENSEN, A.G. JOHNELS, B. NUCCI and T. WESTERMARK (1971). Occurrence of mercury, DDT and PCB in pike from the Stockholm archipelago. (In Swedish) Report to National Environmental Protection board.

TSUKAMOTO, H. et al. (1969). The chemical studies on detection of toxic compounds in the rice bran oils used by the patients of Yusho. Fukuoka Acta med. 60:497-512.

WESTIN, S.I. (1969), reviewed in BERGLUND, F., M. BERLIN, G. BIRKE, R. CEDERLÖF, U. von EULER, L. FRIBERG, B. HOLMSTEDT, E. JONSSON, K.G. LUNING, C. RAMEL, S. SKERFVING, Å. SWENSSON, S. TEJNING (1971). Methyl mercury in fish. A toxicologic-epidemiologic evaluation of risks. Report from an expert group. Nordisk Hygienisk Tidskrift, Suppl. 4:252.

WESTÖÖ, G. and K. NORÉN (1970 a). Determination of organochlorine pesticides and polychlorinated biphenyls in animal foods. Acta chem. scand. 24:1639-1644.

WESTÖÖ, G. and K. NORÉN (1970 b). Levels of organochlorine pesticides and polychlorinated biphenyls in fish caught in Swedish water areas or kept for sale in Sweden. Vår föda 22: 93-146. (Swedish, with summary and tables in English.)

WESTÖÖ, G., K. NORÉN and M. ANDERSSON (1970). Levels of organochlorine pesticides and polychlorinated biphenyls in margarine, vegetable oils, and some food products of animal origin on the Swedish market in 1967-1969. Vår föda 22:9-31.

WESTÖÖ, G., K. NORÉN and M. ANDERSSON (1971). Levels of organochlorine pesticides and polychlorinated biphenyls in some cereal products. Vår föda 23:341-360.

MONS 201060

FOOD EXPOSURES TO POLYCHLORINATED BIPHENYLS

A brief description will be presented of the various incidents that have occurred involving accidental exposure of foodstuffs with PCBs. The main problems in this area relate to sporadic exposures of foods caused by leakage of heat exchange fluid containing PCBs into the animal feeds being processed. To the best of our knowledge the problem of PCBs in heat exchange fluids used in systems used to process food or feed has largely been corrected and an FDA requirement to that effect is to be published shortly in the FEDERAL REGISTER. Other types of contamination of animal feed relates to the practice in some areas of using out-dated bakery products for poultry feed in which the paper containers at times have not been removed from the bakery products prior to processing for feed use. Recycled paper products may contain PCBs in significant amounts but the migration into contained foodstuffs usually is very minimal. Again, a FDA requirement will in effect place further controls upon this potential source of exposure. Fish, primarily from fresh water locations, appear to be the primary source of human exposure through foods. Most states having significant problems with fish contaminated by PCBs have taken appropriate steps to restrict dietary intake by issuing public warnings and imposing other requirements.

Albert C. Kolbye, Jr.

MONS 201061

ABSTRACT

OCCURRENCE OF POLYCHLORINATED BIPHENYLS IN HUMANS

by

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The Human Monitoring Survey was established to determine on a national scale the levels and trends of certain more commonly used pesticide chemicals in the general population. Michigan Department of Public Health is one of several state health laboratories providing residue analyses of human adipose samples which are collected from hospitals scattered across the United States.

Since 1966 this laboratory has been analyzing samples of human adipose tissue routinely by dual-column gas chromatography. During the course of this work a recurring pattern of unidentified peaks was observed on chromatograms, some peaks eluting much later than p,p'DDT. The presence of these unknown compounds complicated the analysis of organochlorine pesticides due to having similar retention times, particularly to p,p'DDT, o,p'DDT, and p,p'DDD.

After Widmark (J. A. O. A. C. 1970) and others reported the presence of polychlorinated biphenyls in animal and environmental media, our attention was focused on this new type of environmental problem. Upon receipt of reference Aroclors from the Pesticides Repository, Perrine Primate Laboratory, E. P. A., this laboratory demonstrated a remarkable similarity between chromatograms of Aroclors, i. e., 1254 and 1260, and those of some human adipose samples.

In this paper, several examples of unusually high levels of polychlorinated biphenyls in human adipose will be presented. Using combined gas chromatography-mass spectrometry, the Perrine Primate Laboratory found that these particular samples contained substantial quantities of polychlorinated biphenyls ranging from pentachlorobiphenyl to decachlorobiphenyl.

Currently this laboratory is conducting semi-quantitative analysis of polychlorinated biphenyls in human adipose tissue by thin-layer chromatography on a total chlorine basis after extracted residues have undergone dehydrochlorination and oxidation steps. According to this analytical method, almost 50% of human adipose samples received by this laboratory contain 1.0 ppm or more polychlorinated biphenyls, using Aroclor 1260 as the reference standard.

MONS 201062

Effect of Aroclor 1254 on Ring Doves and Kestrels.

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and Stephen D. Bloom
(Department of Poultry Science, Cornell University).

The breeding success of Ring Doves(Streptopelia risoria) fed Aroclor 1254 at 10ppm was determined over two generations. Six pairs of birds were fed PCB's for three months, then allowed to incubate their clutch of two eggs. Then eggs were collected again until six months when they were allowed to incubate another clutch. Results are given in table 1. The low hatching success of the 2nd generation was due to heavy embryonic mortality. The age of embryonic death varied considerably, mainly in the range of 3-8 days. This finding is at variance with that of Scott et. al.(1971) who found significant decrease in hatchability of chicken eggs in the first generation with 10ppm Aroclor 1248 for eight weeks and almost complete failure at both four and eight weeks at 20ppm Aroclor 1248. In this case mortality occurred immediately before hatching.

Eggshell thinning was not observed in either the first or second generation(Peakall, 1971; and unpublished data). This is in agreement with the findings of Dahlgren and Linder(1971) for the pheasant(Phasianus colchicus)and Heath et. al. (in press) for the Mallard(Anas platrhynchos) and Lincer (unpublished)for the American Kestrel(Falco sparverius). Some studies were made on the levels of carbonic anhydrase in the oviduct of Kestrels treated with Aroclor 1254(10ppm in diet), DDE(5ppm) or DDE + Aroclor 1254(5ppm and 10ppm respectively). Sample size was small, but values indicate no increase with Aroclor 1254 and decreases with Aroclor 1254 + DDE were equivalent to DDE alone. This finding agrees lack if eggshell thinning caused by PCB's.

Chromosomal studies, using three day embryoes, have been started.

Bloom will : here

NONS 201063

Residue analysis on eggs laid at various times from the start of the experiment showed that the levels increased for three months when an approximately steady xxxxx level of 50 ppm(dry weight) was reached. The second generation eggs had similar levels; thus increased embryonic mortality was not caused by increased levels of PCB's.

The most striking finding of the seabird "wreck" in the Irish Sea in 1969 was the elevated concentration of PCB's in the liver(Holdgate,1971). In birds shot as controls the concentration was only 0.4ppm(wet weight) whereas birds found dead had 40ppm in the liver. In an attempt to evaluate these findings we stressed five first generation birds after they had been exposed to Aroclor 1254 in their diet for six months. The stress was applied by reducing the food intake severely so that the birds lost approximately 10 percent body weight per week. Control,birds were also stressed in a similar manner. No signficant difference was found in either the time to death nor in the weight loss to death between controls and experimental groups. The organ levels of the first generation birds sacrified at the end of the breeding experiment can be compared to organ levels after stravation(table2). A massive increase in all tissues studied as a result of mobilization of fat is clearly shown. The fact that liver levels ranged from 937 - 1688 ppm at the time of death strongly suggests that the 40ppm recorded in the seabirds in the Irish Sea was not responsible for the death of these birds.

Table 1.

	No. of pairs	Eggs laid	Hatched	Fledged
Pre-experiment	6	24	22	22
1st generation	6*	24	24	24**
2nd generation	6	20	4	2
Control, 2nd gen.	6	24	22	22

* One pair failed to produce any eggs during the period of the experiment.

** Fledging losses consisted of one young found outside the nest at one day of age and one deformed bird killed at the age of three weeks.

Table 2.

	Muscle	Brain	Liver	Fat
Pre-stress	8.1 ± 2.3(4) (4.1 - 9.9)	5.5 ± 1.6(4) (4.8-8.0)	15.3 ± 12.6(4) (3.6-27.5)	736.1 ± 311.6 (481.2-1069.4)
Post-stress	172.9 ± 38.3(5) (120.0-227.0)	293.0 ± 27.6(5) (254.0-340.2)	1118 ± 267(5) (937.3-1688.0)	*

Figures are means, standard deviations, sample size and range.

* No fat present.

MONS 201065

POLYCHLORINATED BIPHENYLS: THEIR EFFECTS ON PENNED PHEASANTS[1]

by

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ABSTRACT

In 1970 and 1971, game-farm cock pheasants (Phasianus colchicus) were given single capsules weekly containing 0 and 25 mg polychlorinated biphenyls (PCB), Aroclor 1254, and hens were given 0, 12.5, and 50 mg weekly for 17 and 16 weeks, respectively. Egg production was reduced ($P < 0.01$) in the hens given 50 mg PCB both years. Fertility, determined by visual inspection of incubated eggs, was unaffected by PCB. Hatchability was lowest in the group of hens given 50 mg in both years, but significantly lower ($P < 0.01$) only in 1971. PCB adversely affected the viability of the embryo both years, as the number of eggs pipped but not hatched in PCB groups was higher ($P < 0.05$). PCB did not affect eggshell thickness. PCB-treated birds had fewer feathers than controls.

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[1]Funds for the study were supplied by the Bureau of Sport Fisheries and Wildlife through the South Dakota Cooperative Wildlife Research Unit, supported jointly by the South Dakota Department of Game, Fish and Parks; the Bureau of Sport Fisheries and Wildlife; the South Dakota State University; and the Wildlife Management Institute. Tissue analyses were made by the Denver Wildlife Research Center, and by Dr. Yvonne A. Greichus, Experiment Station Biochemistry Department. Dr. Robert J. Bury, Veterinary Science Department, conducted necropsies and histopathologic studies. Dr. W. L. Tucker, Experiment Station Statistician, gave statistical advice. This work is from a doctoral dissertation in the Animal Science Department by the senior author.

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Behavior on the visual cliff shortly after hatching was affected in that offspring of hens given 50 mg PCB in 1970 ($P < 0.01$) chose the visually-deep side of the cliff. No difference in behavior on the visual cliff between groups was evident in 1971. Response to hand catching of offspring was affected in 1970 ($P < 0.01$); the ability of penned pheasants to avoid hand capture was least where both parents received PCB, followed by those where hens only, cocks only, and neither parent received PCB. No difference in response to hand capture among groups was found in 1971. Weights of chicks from hens on 50 mg PCB were lower ($P < 0.01$) than those of chicks from hens given 0 or 12.5 mg at 6 weeks of age in 1970; this effect was not seen in 1971. Survival of chicks in brooders to 6 weeks of age was lower ($P < 0.01$) in offspring of hens given 50 mg PCB weekly in 1970; no difference was found among offspring of treatment groups in 1971. Survival of young from 6 weeks of age to the fall was similar in all groups. Overall survival from hatching to the fall was less ($P < 0.05$) in offspring of hens given 50 mg weekly in both years combined. No differential effect on survival was found between sexes.

PCB caused hyperexcitability, weakness, tremoring, lack of appetite, and comatose death. Birds 11-weeks old given 10 or 20 mg PCB daily, birds 6-9 months old given 50 or 100 mg each 3.5 days, and adult hens given up to 50 mg once a week continued to eat; 11-week old birds given 210 mg daily stopped eating. Of those given 210 mg daily, the heaviest birds lived longest and lost the greatest percentage of their weight (15-31 percent) before death (1.3-4.3 days). Birds not given PCB, but starved, lost a greater percentage of weight (27-51 percent)

before death and took longer to die (2.3-8.6 days). Of 10 birds given 10 mg daily, one died as early as 30.6 days after the first capsule while one bird of the group lived 8 months and was sacrificed. The ninth bird in this 10-mg group died in 179.3 days, while the other seven birds died between 50.3 and 60.6 days. Of 10 birds given 20 mg PCB daily, one died as early as 39.6 days, and the last bird died 54.1 days after the initial capsule was given. The average number of days to death in the 20-mg group was 46.1 ± 5.3 days standard deviation. Four of 22 birds of both sexes, 6-9 months old, died after 10 single capsules containing 50 mg PCB were given at 3.5 day intervals; 9 of 22 were killed by 10 capsules each containing 100 mg administered similarly. Analysis of brain, liver, and muscle tissues from dead and surviving birds showed that brain residue levels from 300-400 ppm wet weight were indicative of death from PCB toxicosis; liver and muscle levels were too variable to be useful in assessing cause of death.

Adult hens were given a single 50-mg capsule, and samples of whole-body, brain, liver, muscle, eggs, and feces were analyzed. Residue levels of PCB were highest 12 hours after capsule administration; residues were highest in liver, followed by brain and muscle for 28 days after dosage. At least 94 percent of the administered dose was absorbed and 40.5 mg of the 50 mg was in the body after 28 days. Hens were in a state of low egg production, and excreted 4.2 mg in the eggs and 4.0 mg in the feces over 28 days. Excretion in the egg could be an important means of ridding the body of PCB. Eggs from hens given a single capsule containing 50 mg PCB had from 25 to 49 ppm wet weight in their eggs; a single egg laid between the first and second week after treatment contained 1.5 mg PCB. Hens from the 1970 breeding

experiment given 17 capsules weekly and killed 1 week following the last capsule had, for four hens given 12.5 mg per week, from 37 to 56 percent of the administered dose in their body, and, for four hens on the 50-mg level, from 60 to 82 percent of the dose given.

Administration of PCB decreased weights of hearts and spleens ($P < 0.01$) at all levels given and increased weights of kidneys and livers in birds given 10- and 20-mg doses daily ($P < 0.01$), but not in birds given 210 mg daily. Splenic atrophy was characteristic of birds given PCB. Such small spleens were pale-tan color and had wrinkled capsules resulting from depletion of lymphatic nodules.

PCB and dieldrin were given singly and jointly, and only additive, not synergistic, effects were noted. Among the 22 pheasants in each test group given capsules each 3.5 days 10 times, three died from 4 mg dieldrin per capsule, six died from 8 mg dieldrin, four died from 50 mg PCB, nine died from 100 mg PCB, and nine died from 4 mg dieldrin jointly given with 50 mg PCB.

After alternately starving and feeding birds which had PCB stored in their bodies, it was found that PCB levels increased in the brain tissue. This work is presently being prepared for publication.

Toxicity of PCB on non-human mammals and on birds

J.G. Vos

In contrast to the acute toxicity, the subacute and chronic toxicity of PCB is more important. From the literature one can distinguish 2 actions of PCB on mammals, namely liver damage and skin lesions. The former action is predominantly studied in rats, guinea pigs, rabbits, mice and monkeys in feeding and to a lesser extent in inhalation experiments; the latter in rabbits in dermal toxicity studies. Besides liver damage, PCB can also cause 'chick-edema-like' lesions in birds. A similar edema formation is known from the feeding of "toxic fat" to chickens. A toxic factor, responsible for the chick edema, has been isolated and identified: 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. The related compound 2,3,7,8-tetrachlorodibenzo-p-dioxin, which may appear as an impurity in the synthesis of 2,4,5-trichlorophenol and its derivatives such as 2,4,5-trichlorophenoxyacetic acid, is found to be responsible for the skin lesions (chloracne) and liver damage caused by the crude preparations.

Both chloracne and edema formation were noted from technical PCB mixtures. The possibility of contamination of crude preparations with impurities was therefore considered. To clarify this question the following experiments were carried out, using a sample of 3 PCB preparations (containing 60 % chlorine on the average) from different manufactures. A marked resemblance between the gaschromatograms and mass spectra of these mixtures was noted. In a comparative feeding test (400 ppm) in chicks, it was found that high mortality, subcutaneous and abdominal edema, and centrolobular liver necrosis occured in only 2 groups (Clophen A 60 and Phenoclor DP 6). Hydropericardium, a common effect of these 2 samples, was only occasionally seen in the chicks fed Aroclor 1260. Hepatic porphyria (increased excretion of fecal coproporphyrin and protoporphyrin; red fluorescence of tissues in ultraviolet light) was a general PCB effect. The difference in toxicity between the samples was confirmed in a chick embryo assay. By means of column and gaschromatography, the presence of relatively more polar compounds was demonstrated in the 25 % diethylether fractions of the Clophen and Phenoclor samples. By mass spectrometric analysis, the mass numbers and chlorine contents of these compounds were established. From the presence of a similar fragmentation pattern as hexachlorodibenzo-p-dioxin, from the toxicity of the isolated diethylether fraction in eggs, and from pathological data, the structures tetra- and pentachlorodibenzofurans were proposed. They were considered responsible for the higher toxicity of the Clophen and Phenoclor samples.

-2-

These 2 preparations, and their diethylether fractions, caused the most severe skin lesions and liver damage in a dermal toxicity study in rabbits. Kidney lesions were also seen. To evaluate the contribution of impurities in the toxicity of commercial PCB preparations, another dermal toxicity study in rabbits was carried out. Using Aroclor 1260 and 2,4,5,2',4',5'-hexachlorobiphenyl, skin damage due to the latter compound was clearly less severe when compared with the former mixture. Liver injury was somewhat more severe in the 2,4,5,2',4',5'-hexachlorobiphenyl group, though they were essentially the same.
They included centrolobular necrosis and hydropic degeneration. Moreover, by electron microscopy a proliferation of smooth surfaced membranes of the endoplasmic reticulum (SER) was observed. This proliferation can be considered the structural indication for an enhanced metabolism of foreign lipophilic compounds. 2,4,5,2',4',5'-Hexachlorobiphenyl caused increased excretion of fecal porphyrins. The porphyrogenic effect was found to be closely associated with an increase in the activity of mitochondrial δ-aminolevulinic acid (ALA) synthase in quail liver. Aroclor 1260 at a dosage of 1 mg/kg (orally for 1 week in Japanese quail) did increase significantly the activity of this enzyme (mean liver residue 1.41 ppm).
From earlier studies we considered an immunosuppressive action. This was confirmed in an experiment using guinea pigs. Feeding of 10 ppm Aroclor 1260 for 8 weeks resulted in a decreased number of antibody forming cells, after stimulation of the humoral lymphoid system with tetanus toxoid.

From these experiments may be concluded that chlorinated dibenzofurans are responsible for the edema formation; PCB itself for the hepatic porphyria. The liver damage and skin lesions, caused by commercial PCB preparations, come probably for the greater part from chlorinated dibenzofurans contamination, and for a minor part from PCB itself. A proper evaluation of toxicity and residue data can be hindered by a contamination of PCB with toxic impurities.

TOXICOLOGICAL STUDIES WITH

POLYCHLORINATED BIPHENYLS

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MONS 201072

In 1966 (Sweden) and in 1967 (USA) polychlorinated biphenyls (PCB's) were detected in certain birds and in other samples taken from the environment. Since that time, numbers of other reports have been published which indicate that the PCB's have been found in other samples of fish, birds, and water.

Polychlorinated biphenyls are manufactured in this country under the name Aroclors by the Monsanto Company and in Europe and Japan under such trade names as Phenochlor and Clophen. Aroclors are manufactured by replacing one or more of the 10 hydrogen atoms in biphenyl with chlorine atoms. Theoretically, there are about 210 compounds which can be made by different substitutes. Biphenyl is chlorinated to a certain percent of the molecular weight. Thus, an Aroclor is a mixture of compounds, not a single chemical entity.

There was virtually no data in 1966 to indicate possible toxicological hazard from long term ingestion or exposure to small quantities of the PCB's. Therefore, a number of toxicity studies was started.

The polychlorinated biphenyls (PCB's) investigated were Aroclor 1242 (biphenyl chlorinated to 42% by weight), Aroclor 1254 (chlorinated to 54%) and Aroclor 1260 (chlorinated to 60%). These compounds are not very toxic acutely. The oral LD_{50}'s in the rat are about 2 to 10 g/kg.

Preliminary (30-day) feeding studies in rats were conducted. When fed at 1000 ppm in the diet, there were decreases in food consumption and rate of gain of body weight. At 300 ppm in the diet, there was no apparent effect from Aroclor 1254 or 1260, but there were slight (but not statistically significant) decreases in food consumption and rate of weight gain from 1242.

More extensive studies were then started. These included two-year feeding studies in rats and dogs, three-generation reproduction studies in rats, reproduction studies in chickens and teratogenic studies in rats. Feeding levels selected were 1, 10 and 100 ppm. The two-year rat feeding study was started with sufficient animals for interim sacrifices at 3, 6 and 12 months. Seventy animals per group (35 males and 35 females) were fed the full two-year period. A two-year dog feeding study was started with eight dogs (four males and four females per group). In both of the two-year studies, body weights, food consumption, hematology, clinical chemistry studies (BUN, glucose, SAP, SGPT and SGOT), organ weights, histology of 25 to 30 organs and tissues, and the saving of frozen tissues for chemical analyses were included.

In the rat studies, after 24 months, the liver weights and liver to body weight or brain weight ratios were significantly elevated in animals fed 100 ppm of the three Aroclors. Histologic examination of the livers from the groups fed 100 ppm revealed several animals

with vacuolar change, indicative of fatty degeneration. Specific fat stains confirmed the presence of fat in these vacuoles. Focal hypertrophy and focal hyperplasia were also found in the livers from some of these animals. All other parameters at 100 ppm and all parameters at 1 or 10 ppm were normal.

There were no effects in dogs fed Aroclor 1242 or 1254 for 24 months at 1, 10 or 100 ppm. Dogs fed Aroclor 1260 at 100 ppm showed increased serum alkaline phosphatase activity and increased liver weights. At 1 or 10 ppm of Aroclor 1260, no effects were observed in the dogs.

In the three-generation studies weanling rats were fed the compounds in the diet until 100 days of age, at which time they are allowed to mate. Pups from the second litter were used as parents of each succeeding generation. Mating indices, fertility indices, incidences of pregnancy and incidences of parturition were recorded for parental animals, as well as body weights, reactions and pathology. Live birth indices, survival indices (at 24 hours and 5 days) and lactation indices for progeny were calculated.

There were low mating indices and decreased survival of pups at 100 ppm Aroclor 1242 and decreased survival of pups at 100 ppm Aroclor 1254. There were no effects at 1 or 10 ppm Aroclor 1242 or 1254 or at 1, 10 or 100 ppm Aroclor 1260.

White Leghorn chickens were fed Aroclor 1242, Aroclor 1254 or Aroclor 1260 at 1, 10 or 100 ppm in the diet. Effects on the chickens as well as on egg production, hatchability of the eggs and physical characteristics of the eggs were determined.

The body weights of males fed Aroclor 1242 at 10 or 100 ppm and of males and females fed Aroclor 1254 at 100 ppm were slightly lower than the weights of control chickens. Their food consumption also was low. Some birds died showing evidence of malnutrition.

Egg production in hens fed Aroclor 1242 at 100 ppm or Aroclor 1254 at 100 ppm was much lower than the egg production of the control group. There was poor hatchability of eggs from hens fed Aroclor 1242 at 10 ppm. None of the eggs hatched from hens fed Aroclor 1242 at 100 ppm or Aroclor 1254 at 100 ppm.

There were no significant differences in specific gravity among eggs from treated or from control hens. The egg shells, however, from hens fed Aroclor 1242 at 10 or 100 ppm or fed Aroclor 1254 at 100 ppm were not as thick as the shells of eggs from hens fed the control diet.

Therefore, the ingestion of Aroclor 1242 at 1 ppm, Aroclor 1254 at 10 or 100 ppm or Aroclor 1260 at 1, 10 or 100 ppm caused no apparent effects on the mature chickens or on their eggs, including hatchability and physical characteristics of the eggs. However, the chickens fed Aroclor 1242 at 10 or 100 ppm or Aroclor 1254 at 100

ppm did not produce as many eggs as the chickens fed the control diet. The eggs from these three groups of chickens had thin shells and did not hatch well after incubation.

An additional reproduction study in chickens was conducted with 1242 at levels of 2, 4 and 8 ppm. In general, there were no effects attributable to Aroclor 1242 with the possible exception of slightly decreased hatchability at 8 ppm.

Teratogenicity studies in rats were conducted with all three Aroclors. The oral dosage levels were 10 and 30 mg/kg/day. Administration was from day 6 through day 15 of gestation. The results showed no evidence of teratogenic effects.

Epidemiologic study on "Yusho", a poisoning caused by rice oil contaminated with polychlorinated biphenyls

by

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MONS 201078

Sporadic outbreaks of a peculiar skin disease which was later called as "Yusho" were first reported in Fukuoka-Ken (Fukuoka prefecture), Japan, in early October 1968. It was characterized by such symptoms as follicular accentuation, acneform erruption, pigmentation of the skin and nails, hypersecretion of the Meibomian gland, increased sweating at palms, feeling of weakness, and numbness in limbs. The epidemic spread not only over Fukuoka-Ken but also over 20 other prefectures in the western part of Japan, producing about 1,000 patients. In order to clarify the cause of the epidemic we conducted an epidemiologic investigation of 325 patients seen in Fukuoka-Ken from October 1968 until January 1969, under a close cooperation with the health departments.

1. Descriptive epidemiologic study

Ninety-nine percent of the patients were affected during 1968 and 55 percent of the cases were concentrated from June to August. The geographical distribution of the patients as observed by calculating crude incidence rates did not indicate any common socioeconomic or environmental factor which might be connected with the disease. A distinct familial aggregation was noted because the 325 patients belonged to 112 families. Both sexes, irrespective of age, were shown to be equally affected.

2. Analytical epidemiologic studies.

When our study started, a brand of rice oil produced by

MONS 201079

K company (abbreviated as K rice oil) had vaguely been suspected as a possible cause of the disease. Therefore, a thorough investigation was undertaken to determine whether the patients with Yusho had actually used such brand of oil before affected. It was soon disclosed that all the patients had used K rice oil, either canned or bottled. Furthermore, an astonishing fact was shown that, no matter where they lived, 166 of the 170 patients who used the canned oil had used a very specific oil produced or shipped by the K company on February 5 and 6, 1968 and 143 of the 155 patients who used the bottled oil exclusively might have bought the oil shipped from the company in the period February 6 to 15, 1968. Thus, nearly all of the patients had had a very peculiar common experience of use or possible use of a rice oil produced or shipped by one company in a specific period. The attack rate was as high as 63.9 percent for those who consumed the specific canned oil.

In an additonal survey, we could demonstrate that a group of 113 persons who regularly used K rice oil but did not use the specific K rice oil produced or shipped in the period in question were free of the disease.

In order to know whether any other factors than the rice oil might be the cause of the epidemic, we also made two case-control studies by home interviews. In one, the personal backgrounds of the patients and of their matched controls were compared, while in the other, the use of oils and fats in the patient households and in the control households were compared.

MONS 201080

In the former comparison, only one of 60 personal factors examined, namely habit of "eating fried foods or tempura nearly every day", was significantly more commonly seen among the patients than among the controls. In the latter investigation, a distinct and significant difference was noted between the two groups only for use of rice bran oil. About 96 percent of the patients households had used rice bran oil regularly while only 31 percent of the control households had. When asked about the use of K rice oil, all of the patients households affirmed it. Thus, the case-control studies clearly indicated that none of the factors tested except use of K rice oil could account for the disease.

3. Dose-response relationship

A rough estimation of the quantity of the specific K rice oil consumed by each patient and his family members was made disregarding their age, sex, food intake, and possible loss of oil during and after cooking. A definite dose-response relationship was noted in attack rate as well as in clinical severity.

Thus, all the above epidemiologic findings proved that ingestion of the K rice oil produced or shipped in the specific period of time is the cause of the epidemic.

4. Toxic agent

The chemical study group of the disease (Chief: Prof. H. Tsukamoto, Kyushu University) discovered that the canned K

rice oil produced or shipped on February 5, 1968 and used by some of the patients had been contaminated with about 2,000 ppm of Kanechlor 400, a brand of polychlorinated biphenyls, which is considered to have leaked from the heating pipe of the oil in the process of production. Skin fat collected from the patients also contained Kanechlor. Gaschromatographic analysis of 109 samples of the bottled K rice oil proved that a significant contamination of the bottled oil was limited only to those shipped between February 7 to 10, 1968. Thus, the results of chemical analysis perfectly coincided with those of epidemiologic approaches.

The minimum total dose of Kanechlor 400 to cause the disease was estimated to be around 0.5 g.

5. Other observations

Thirteen women consisting of 10 with Yusho and two unaffected and one status-unspecified wives of patients were shown to have delivered 10 live born and two stillborn infants during the specified period. These infants and fetuses had grayish darkbrown stained skin, nail, and gingiva, and an increased eye-discharge at birth. As they grew older, their stained skin gradually faded. The majority of the babies were small for their periods of gestation.

The affected school children were compared with matched unaffected children for growth. The gains of the affected boys in height and weight was shown to have decreased after the

MONS 201082

poisoning, while the affected girls showed no definite change in this respect.

Many patients with Yusho are still tortured by the disease even after more than 3 years. To cure them and to prevent another epidemic of Yusho, all possible world-wide cooperative efforts are highly desired.

CHLORINATED AROMATIC HYDROCARBON INDUCED MODIFICATIONS OF THE HEPATIC ENDOPLASMIC RETICULUM: CONCENTRIC MEMBRANE ARRAYS

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Ingestion of certain chlorinated aromatic hydrocarbons, including chlorinated triphenyls, chlorinated biphenyls, and dichlorodiphenyltrichloroethane (DDT), and chlorinated dibenzo-p-dioxins, caused marked hypertrophy of the liver of experimental rats and monkeys. When the hepatocytes of these affected livers were examined ultrastructurally and biochemically, there was found to be an increase in lipids and a decided proliferation of the endoplasmic reticulum (ER). Parallel cisternae of the rough ER commonly present in control hepatocytes were reorganized into sinuous patterns that encompassed mitochondria, lipid droplets, and other organelles. Numerous annular profiles of the ER formed from a fusion of these curved cisternae. As a result of the increase in the number of cisternae that encompassed these annular profiles they became progressively larger. Within three weeks, approximately 60% of the hepatocyte profiles contained large concentric membrane arrays. This sequential reorganization of the rough ER and the continuity of the arrays with the

smooth and rough ER led to the conclusion that the concentric membrane arrays originate from cisternal elements.

Associated with the modified membrane structures were alterations in enzyme activity and biochemical composition of the microsomal fraction of these cells. Following the ingestion of these chlorinated hydrocarbons, there was an increase in microsomal protein per gram of liver (54.5 mg. vs. 20.1 mg.). Compositional changes of the membranes were indicated by reduction in cholesterol per mg. of microsomal protein (17.9 ug. vs. 44.0 ug.) and a slight increase in phospholipid per mg. microsomal protein (508 ug. vs. 455 ug.) The increase in esterase activity was parallel to that of protein whereas elevations in N-demethylation and nitro-reduction were greater than the increase in membranous material. Specific activities of glucose-6-phosphatase and aromatic hydroxylase were reduced.

Proliferation of the ER in response to chlorinated biphenyls and triphenyls resulted in the formation of new membranes which are distinct in their morphology, enzyme content, and composition. The proliferation of the ER is apparently more complex than a non-specific increase in membranous material. There are changes in enzyme composition, an increase in phospholipid/protein, and a decrease in cholesterol/protein of the microsomes. Increased enzyme activity, unaltered phospholipid/protein and increased cholesterol/protein have been observed in proliferated smooth ER produced by a wide variety of agents including phenobarbital. Thus membranes low in cholesterol and high

in phospholipids which are characteristic of chlorinated aromatic hydrocarbon induced microsomes likely represent the concentric membrane arrays.

Modification in the biochemical composition of the membranes are likely favorable for certain functional properties. Consistent with the increased proportion of phospholipids within the membranous structure is the proposal that the proliferated membrane system provides a site for localization of the hydrophobic chlorinated aromatic hydrocarbons. The phospholipid may enhance this suitability of membranes for sequestration of the foreign material or for approximation of the compound to the enzymes of the membrane. The proximity of the concentric membranes to lipid droplets also a probable location of the lipid soluble chlorinated aromatic hydrocarbons provides accessibility of the lipid components of the concentric membranes to the compound. Cholesterol within the membrane structure has been related to rigidity of the membrane by its ability to condense the saturated fatty acids of the phospholipids. Lack of cholesterol suggests a less stable structure, or alternatively, regions available for insertion of other compounds such as the chlorinated aromatic hydrocarbons among the fatty acids of the phospholipids.

BIOLOGICAL EFFECTS OF POLYCHLORINATED BIPHENYLS IN RATS AND QUAIL

Joel Bitman, Helene C. Cecil and Susan J. Harris
USDA, ARS, Beltsville, Md. 20705

Polychlorinated biphenyls (PCB's) have been administered to rats and to quail to discover the nature of the biological effects which these environmental pollutants produce in mammals and birds. Biological effects evaluated were (1) estrogenic activity of the PCB's in rats, (2) alterations of pentobarbital metabolism induced by the PCB's as judged by sleeping time of Japanese quail, (3) alterations of liver vitamin A, liver lipids and liver weight induced by Aroclor 1242 in rats and quail, and (4) egg production of Japanese quail on a diet containing Aroclor 1242.

Estrogenic activity was assessed by determining the stimulation of the uterine glycogen response of the immature rat uterus. In a series of 11 polychlorinated biphenyls and terphenyls, the compounds containing up to 48% chlorine were estrogenically active.

A sleeping time test with pentobarbital was utilized as a means of determining liver microsomal enzyme activity since the liver is the major site for the metabolism of barbiturates. The PCB's were tested for their acute or short term effects (48 hours) and for longer term effects at 3 and 7 days. A single dose of Aroclor 1268 or Aroclor 5460 initially increased sleeping times in Japanese quail but by 18 hours sleeping times were less than untreated control values. By 48 hours the PCB's had stimulated the liver enzymes which metabolize pentobarbital and sleeping times were only 50% of control sedation times. Continuous feeding for 3 and 7 days confirmed this, sleeping times being about 50-70% of control levels, thus indicating greater metabolism of the barbiturate.

Aroclor 1242 at a dietary level of 100 µg/g feed was fed to male and female rats and quail for two months. In both rats and quail, liver vitamin A was depressed about 50% but liver weight and liver lipid increased significantly. Average egg production in the Aroclor 1242-fed quail declined to about 71% as compared to 85% in the controls. The number of membranous and broken eggs in the PCB group was over 2X that of the control quail.

MONS 201087

PCB AND INTERACTIONS WITH INSECTICIDES

E. Paul Lichtenstein
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PCB - Meeting, NIEH, Research Triangle Park
December 21, 1971

ABSTRACT

The effects and interactions of environmental chemicals on human cells in tissue culture were studied by determining the toxicity of some of these chemicals on HeLa cells and skin fibroblasts. The polychlorinated biphenyl plasticizer (Aroclor 1254), carbaryl, parathion, DDT, several insecticide metabolites, aspirin and caffeine were studied. The dosage causing a 50% inhibition in culture growth was determined and the interaction between these chemicals and their effect on protein and nucleic acid synthesis was determined. Both cell types responded nearly equally to the presence of an individual chemical. Aspirin and caffeine were 8 to 20 times less toxic than the other chemicals studied, while Aroclor 1254 was as toxic as DDT. Parathion was more toxic than its metabolite paraoxon. Para-nitrophenol was as toxic as parathion. 1-Naphthol was less toxic than its parent compound carbaryl. The toxicities of DDT and DDE were dependent upon cell type. Little significant interaction on nucleic acid or protein synthesis was seen (1).

MONS 201088

The biological interaction between plasticizers and some insecticides was also investigated utilizing insects as the test objects.

Because of the structural similarity to DDT-related compounds, 11 plasticizers were tested for their toxicological interaction with dieldrin and DDT. Many of these polychlorinated biphenyl compounds were toxic to Drosophila melanogaster Meigen and house flies, Musca domestica L., but to a lesser extent than dieldrin or DDT. Their toxicity increased with a decrease in their chlorine content. Moreover, sublethal dosages of several of the plasticizers increased the toxicity of dieldrin and DDT (2).

In particular, though, a substantial increase in the toxicity of organophosphorus insecticides to houseflies was achieved with polychlorinated biphenyl plasticizers. Aroclor 1248 -- although non-toxic by itself to house flies at dosages of 10 microgram per fly -- significantly increased the toxicity of the oxygen analogs of five organophosphorus insecticides after simultaneous topical application to houseflies. Both p,p'-DDT and p,p'-DDE, applied at sub-lethal dosages, also increased the mortality of houseflies due to paraoxon from 2 to 60 and 73 per cent, respectively. The polychlorinated biphenyl plasticizer, applied simultaneously with parathion, however, decreased the toxicity of parathion to houseflies, but increased mortalities when applied to flies one-half hour after the application of the insecticide. The plasticizer apparently inhibited the conversion of parathion to its toxic activation product, paraoxon, and the breakdown of paraoxon into non-toxic compounds. This was also indicated by the recovery of larger amounts of ^{14}C-paraoxon and smaller amounts of water soluble ^{14}C-products from flies treated simultaneously with Aroclor 1248 plus paraoxon

in comparison to those which had been treated with ^{14}C-paraoxon only. Utilizing the 10,000 x G supernatant from housefly homogenates, the addition of Aroclor 1248 with ^{14}C-paraoxon in vitro also resulted -- after 2 hours incubation at 30°C -- in higher recoveries of ^{14}C-paraoxon and in a reduction of the appearance of water soluble ^{14}C-products. Identical results were obtained with subcellular fractions (10,000 x G supernatant) from flies that had been treated with Aroclor 1248 eighteen hours before homogenate preparation. Aroclor does not appear to inhibit the synthesis of paraoxon degrading enzymes since no difference was observed in the degradation of paraoxon or the appearance of water soluble products with Aroclor applied to the living flies or to the 10,000 x G supernatant of homogenates from untreated flies(3).

Since plasticizers are in the environment, their potential effects on biological systems, especially in combination with other synthetic chemicals, should not be disregarded.

(1) Litterst, C. L. and E. P. Lichtenstein. 1971. Effects and interactions of environmental chemicals on human cells in tissue culture. Arch. Envir. Health 22: 454-59.

(2) Lichtenstein, E. P., K. R. Schulz, T. W. Fuhremann and T. T. Liang. 1969. Biological interaction between plasticizers and insecticidea. J. Econ. Entomol. 62(4): 761-65.

(3) Fuhremann, T. W. and E. P. Lichtenstein. 1972. Increase in the toxicity of organophosphorus insecticides to houseflies due to polychlorinated biphenyl plasticizers. J. Toxicol. & Appl. Pharmacology (submitted).

MONS 201090

POLYCHLORINATED BIPHENYL INTERACTIONS WITH TISSUE CULTURE CELLS

Roger Hoopingarner, Albert Samuel, David Krause
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ABSTRACT

Polychlorinated biphenyls were tested for cytological effects against established tissue culture lines and primary human lymphocyte cell cultures. Cytotoxicity increased inversely to the percentage chlorine. This correlation was confirmed in the distillation fractions prepared either commercially or in the laboratory. Synergistic studies with DDT indicate similar but only additive action. Cells also react differently to the concentrations of PCB's under different stages of growth or stress.

Cell cycle studies were conducted to evaluate chemical effects at the various stages of the mitotic cycle. Since the lymphocyte cultures have a long inactive stage before initiation of the cycle, the effect on this differentiated (G_0) state was also tested. The changes in growth (mitotic index) as well as chromosome aberrations were evaluated for selected PCB's applied during the cell cycle stages G_1, S (DNA synthetic), and G_2 plus mitosis in both human lymphocyte cultures and chinese hamster cell lines.

MONS 201091

PCB Meeting - December 20-21, 1967

Dr. David Stalling, Chemist and
Dr. Foster L. Mayer, Jr., Fishery Biologist
Fish-Pesticide Research Laboratory
Columbia, Missouri

Abstract

Toxicities of PCBs to Fish and Environmental Residues in Fish

PCB residues were exsmined by GC and GC-MS in fish taken from selected river systems. Silicic acid column chromatography and gel permestion chromatography were used for sample cleanup. Four fish samples from the Ohio River contained an average of 80 µg/g total PCBs (1232, 1248, 1254, and 1260). The highest PCB residues occurred in a sample from the Hudson River (213 µg/g total). DDT/PCB ratios in the twelve highest of 40 fish samples ranged from 1.3 to 211. High ratios were associated with heavily industrialized river systems.

The acute toxicities (96-hr LC_{50}) of PCBs to fish range from 278 to 50,000 µg/l while that for aquatic invertebrates is 3.2 to 2,400 µg/l. The LC_{50} values for fish exposed for 10 to 30 days in continuous flow diluters varied from 8 to 151 µg/l and 5 to 10 day exposures to invertebrates resulted in LC_{50} values of 0.6 to 80 µg/l. The toxicity of Aroclors 1242-1260 were inversely related to percent chlorination. The acute oral toxicity of Aroclors 1242-1260 to fish was greater than 1500 mg/kg. Biological magnification of Aroclors 1248 and 1254 in fish were 6,300 to 71,400 times the exposure levels after 60 days and 1254 was magnified 12,600 to 45,000 times in aquatic invertebrates.

MONS 201092

The Sensitivity of Fish ATPases to Polychlorinated Biphenyls

Presented by L. K. Cutkomp
Department of Entomology, Fisheries, & Wildlife,
University of Minnesota, St. Paul

In spite of widespread concern about the possible hazards of polychlorinated biphenyls (PCBs), little is known about the action of these materials. We have therefore examined the *in vitro* and *in vivo* effects of PCBs on the ATPase enzyme system. We have found some effects which are similar, yet distinctive, to chemically-related compounds of the DDT type. In earlier research we have shown inhibition of Mg^{2+}ATPase and Na^{+}-K^{+}ATPase.

Tissues from blue gill fish, *Lepomis machrochirus*, were dissected, homogenized, and fractionated by centrifuging at 13,000 g for 20 minutes, and the sediments were resuspended in 0.32 M sucrose, 1 mM EDTA, and 10 mM imidazole. The fraction obtained (B) contained mitochondrial and nerve ending particles. Each preparation was appropriately diluted and the samples were quick frozen in liquid nitrogen and stored at -20° C until the ATPase assay. ATPase activity was determined using the enzymatic or continuous procedure in which catalysts pyruvate kinase and lactic dehydrogenase were used and NADH is oxidized to NAD for spectrophotometric determination at 340 nm. Mg^{2+}ATPase activity was measured when 1 mM ouabain was in the reaction mixture. Ouabain is a cardiac glycoside which specifically inhibits the Na^{+}-K^{+}ATPase. Mg^{2+}ATPase was further separated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive portions by adding 0.03 µg oligomycin (oligomycin A 15%, B 85%) per ml reaction mixture.

MONS 201093

Four PCB preparations (Aroclor 1221, Aroclor 1242, Aroclor 1254 and Aroclor 1268) and a polychlorinated terphenyl, Aroclor 5460 were used in these investigations. Aroclors 1221, 1242 and 1254 were dissolved in ethanol and Aroclors 1268 and 5460 in acetone and added to the reaction mixture by slowly releasing the solution from a Hamilton micro-syringe under the liquid surface of the vortex of a rapidly stirred reaction mixture. Reaction temperature was 37°C, except for muscle where the temperature was maintained at 27°C. The amount of solvent added with the PCBs had no effect on the ATPase activities.

Four tissues of fish (brain, muscle, liver and kidney) were used to test the effects of the PCBs. Oligomycin - insensitive Mg^{2+}ATPase from muscle homogenate was most sensitive in in vitro tests. Aroclors 1242 and 1254, in the intermediate range of chlorination, were more effective than 1221 and 1268. Compared at 50% inhibition the values were 0.6 ppm for Aroclor 1242 and 1.2 for Aroclor 1254. Mg^{2+}ATPase from mitochondria (oligomycin-sensitive) was not as sensitive to the PCBs as to DDT-type compounds. The I_{50} for Aroclor 1242 was 2.0 ppm on mitochondrial Mg^{2+}ATPase.

Some stimulation of Mg^{2+}ATPase was evident from the poorest inhibitors, Aroclors 1221 and 1268. Na^{+}-K^{+}ATPase from fish brain homogenate was inhibited by Aroclor 1242 but the dose required was several times (>6x) that for Mg^{2+}ATPase.

The in vivo studies were conducted with fat head minnows, Pimephales promelas which were chronically exposed to Aroclor 1242 and 1254 for 4 months. Comparisons of ATPase activity showed some inhibition and some stimulation over a concentration range in which fish were exposed to 0.31, 0.93, 2.8 and 8.3 ppb. Aroclor 1242 showed the greatest inhibition of Mg^{2+}ATPases

both in vitro and in vivo and its greatest effect was in kidney tissue. A maximum inhibition of Aroclor 1242 in treated fish (in vivo) was 75% on mitochondrial Mg^{2+}ATPase and 36% on Na^{+}-K^{+}ATPase. In contrast, Aroclor 1254 did not inhibit mitochondrial Mg^{2+}ATPase in kidney, but in one group treated with 8.3 ppm inhibited 30% Na^{+}-K^{+}ATPase in kidney. Somewhat more consistent results showing inhibition were obtained from brain tissues.

Overall, PCB treated fish showed more variation of effects on the ATPases, therefore the precision of the results obtained in vitro could not be duplicated in in vivo studies. However, the greatest effects were on Mg^{2+}ATPase, with lesser effects on other components of the ATPase system. Aroclors in the intermediate range of chlorination appeared to have the greatest inhibitory effect, especially Aroclor 1242.

DDT AND AROCLOR 1221 (Monsanto) DISRUPT OSMOREGULATION IN MARINE ADAPTED EELS (Anguilla rostrata) and KILLIFISH (Fundulus heteroclitus). William B. Kinter, Louise S. Merkens, Ralph H. Janicki, and Anthony M. Guarino. Mt. Desert Island Biol. Lab., Salisbury Cove, Me. 04672; SUNY Upstate Med. Ctr., Syracuse, N.Y. 13210; Natl. Cancer Inst., Bethesda, Md. 20014.

Ability to osmoregulate was followed in individual eels (about 100 g) placed in 15 C sea water (2 liters) containing 0.1% ethanol and 1 ppm DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane], a level which is lethal by 10 hr. At 6 hr, plasma milliosmolality averaged 462 ± 10 SE (5 fish) compared to 358 ± 10(5) for ethanol controls and much of this large increase was due to plasma Na. In additional experiments with ^{14}C-DDT, the fish had taken up about half the radioactivity by 6 hr and the ppm in intestinal mucosa and gill filaments averaged 7 ± 1(6) and 22 ± 2(6), respectively, on a wet weight basis. Using isolated eel gills incubated 1 hr in 15 C, oxygenated, artificial, sea water (M. Kamiya, Annot. Zool. Japon. 40:123, 1967), the fractional ^{22}Na space was increased significantly ($P<0.01$) from 0.11 ± 0.00 (15 gills) to 0.16 ± 0.01(15) by 50 ppm DDT solubilized in the water with 0.5% N,N-dimethylformamide (DMF). Using eel gill homogenate and our assay procedure (Janicki and Kinter, Nature 233:148, 1971), the Na, K-activated ATPase was inhibited 38% ($P<0.01$) by 50 ppm DDT solubilized with 5% DMF. These and our previous data, including that from eel intestine (Janicki and Kinter, Science 173:1146, 1971), support the view that DDT inhibits Na transport in the primary osmoregulatory organs of marine teleosts. Finally, experiments with killifish (10 fish at 5 g) in 15 C sea water with 0.1% ethanol (2 liters) demonstrated roughly equivalent lethality (24 hr) for 0.25 ppm DDT and 75 ppm Aroclor 1221 (industrial mixture of polychlorinated biphenyls, PCBs, kindly provided by Monsanto Co.); 6-hr exposure to these levels increased ($P<0.05$) plasma milliosmolality from 365 ± 3 (6 samples, each from 3-5 ethanol control fish) to 389 ± 9(8) and 396 ± 7(9), respectively. These results suggest similar mechanisms of toxicity for DDT and PCBs in this marine adapted fish. (Supported in part by NIH Grants AM06479 and GM00293.)

Abstract: NIEHS meeting on PCBs, Dec. 1971.

MONS 201096

ABSTRACT

TITLE: Polychlorinated Biphenyl (Aroclor 1242): Effects of Uptake on E. coli Growth

Authors: J.E.Keil, M.S., C.D.Graber,Ph.D., L.E.Priester,Ph.D. S.H. Sandifer, M.D.

PCB at .01 and .1 ppm consistently stimulated E. coli growth in vitro. Differences between treatments and controls were statistically significant at the 95% confidence level. There was no difference in nucleic acids content between treatments and controls at the conclusion of 24 hours incubation although increased uridine uptake was noted in all PCB cultures after five hours of incubation.

Address of senior author: Julian E. Keil, Associate in Preventive Medicine, Preventive Medicine Section, Medical University of South Carolina, 80 Barre Street, Charleston, S.C. 29401.

MONS 201097

ABSTRACT

Results of a Survey of Agricultural Research on PCB's

Questionnaires concerning PCB research were sent to State Agricultural Experiment Stations in the 50 States and the Agricultural Research Service of USDA. Description of approximately thirty PCB research programs representing approximately 10 scientist man years have been received to date.

The results of the survey relate to research concerned with the toxicology of PCB's enalytical methodology, movement and biological magnification, degradation, environmental contamination in soil, air, water and sewage, and contamination of raw agricultural commodities.

USDA-CSRS
R. C. Riley
12/16/71

MONS 201098

National Institute of Environmental Health Sciences

Meeting on Polychlorinated Biphenyls (PCBs)

Quail Roost Conference Center

Rougemont, North Carolina

December 20-21, 1971

MONDAY, DECEMBER 20

8:50- 9:00	Welcoming Remarks	David P. Rall
	SESSION I: Chemistry	Chairman: Lawrence Fishbein
9:00- 9:30	*Chemistry - General Summary* *J. William Cook*	
9:30- 9:40	*Discussion*	
	SESSION II: Environmental Aspects	Chairman: J. Clarence Davies
9:40-10:00	*Photochemical Behaviour of Chlorobiphenyls (PCB)* *O. Hutzinger* and S. Safe*	
10:00-10:05	*Discussion*	
10:05-10:25	*Break*	
10:25-10:45	*Rates and Routes of Transport of PCBs in the Environment* *Adel F. Sarofim* and Ian C. T. Nisbet*	
10:45-10:50	*Discussion*	
10:50-11:10	*Accumulation of Polcyhlorinated Biphenyls in Ecosystems* *Robert W. Risebrough* and Brock de Lappe*	
11:10-11:15	*Discussion*	
11:15-11:25	*Contamination of the Bay of Fundy-Gulf of Maine Area with Polychlorinated Biphenyls, Polychlorinated Terphenyls, Chlorinated Dibenzodioxins, and Dibenzofurans* *V. Zitko,* O. Hutzinger and P. M. K. Choi*	
11:25-11:35	*Chlorobiphenyls (PCBs) in Wisconsin Natural Waters* *Gilman D. Veith*	
11:35-11:45	*Discussion*	

*Speaker

MONS 201099

11:45-11:55 *Polychlorinated Biphenyl Residues in the Milk of Environmentally and Experimentally Contaminated Cows*
G. F. Fries

11:55-12:05 *Polychlorinated Biphenyls in Non-carbon Copying Paper*
Masanori Kuratsune and Yoshito Masuda*

12:05-12:15 *PCB and the Paper Industry -- A Progress Report*
Paul Trout

12:15- 1:45 *Lunch*

1:45- 2:05 *Levels of Polychlorinated Biphenyls (PCB) in Swedish Foods*
Fredrik Berglund

2:05- 2:15 *Discussion*

2:15- 2:35 *Occurrence of Polychlorinated Biphenyls in Humans*
Harold A. Price

2:35- 2:45 *On the Incidence of PCBs in Humans*
Anne R. Yobs

2:45- 3:00 *Discussion*

3:00- 3:20 *Break*

3:20- 3:40 *Food Exposures to Polychlorinated Biphenyls*
Albert C. Kolbye, Jr.

3:40- 3:50 *Discussion*

SESSION III: Toxicology Chairman: Albert C. Kolbye, Jr.

3:50- 4:00 *Polychlorinated Biphenyls: Their Effects on Penned Pheasants*
Robert B. Dahlgren, Raymond L. Linder and C. W. Carlson*

4:00- 4:10 *Effect of Aroclor 1254 on Ring Doves and Kestrels*
David B. Peakall, Jefferey L. Lincer and Stephen E. Bloom*

4:10- 4:30 *Toxicity of PCB on Non-human Mammals and on Birds*
J. G. Vos

4:30- 4:35 *Discussion*

4:35- 4:55 *Toxicological Studies with Polychlorinated Biphenyls*
M. L. Keplinger, Otis E. Fancher, J. C. Calandra and Elmer P. Wheeler*

*Speaker

4:55- 5:00 *Discussion*

5:00- 5:20 *Epidemiologic Study on "Yusho", a Poisoning Caused by Rice Oil Contaminated with Polychlorinated Biphenyls*

Masanori Kuratsune, Takesumi Yoshimura, Junichi Matsuzake and Atsuko Yanaguchi*

5:20- 5:30 *Discussion*

6:00- 7:00 *Social Hour*

/:00 *Dinner*

TUESDAY, DECEMBER 21

SESSION IV: Biochemical Actions Chairman: Edward J. Burger, Jr.

9:00- 9:20 *Chlorinated Aromatic Hydrocarbon Induced Modifications of the Hepatic Endoplasmic Reticulum: Concentric Membrane Arrays*

*D. H. Norback and J. R. Allen**

9:20- 9:30 *Biological Effects of Polychlorinated Biphenyls in Rats and Quail*

Joel Bitman, Helene C. Cecil and Susan J. Harris*

9:30- 9:40 *Discussion*

9:40-10:00 *PCB and Interactions with Insecticides*

E. Paul Lichtenstein

10:00-10:20 *Polychlorinated Biphenyl Interactions with Tissue Culture Cells*

Roger Hoopingarner, Albert Samuel and David Krause*

10:20-10:35 *Discussion*

10:35-10:55 **Break**

10:55-11:05 *Toxicities of PCBs to Fish and Environmental Residues in Fish*

David Stalling and Foster L. Mayer, Jr.*

11:05-11:20 *The Sensitivity of Fish ATPases to Polychlorinated Biphenyls*

L. K. Cuthomp

11:20-11:30 *DDT and Aroclor 1221 (Monsanto) Disrupt Osmoregulation in Marine Adapted Eels (Anguilla rostrats) and Killifish (Fundulus heteroclitus)*

William B. Kinter, Louise S. Merkens, Ralph H. Janicki and Anthony M. Guarino*

*Speaker

11:30-11:50 *Discussion*

11:50-12:00 *Polychlorinated Biphenyl (Aroclor 1242): Effects of Uptake on E. coli Growth*
J. E. Keil, C. D. Graber, L. E. Priester and S. H. Sandifer*

12:00-12:10 *Discussion*

12:10- 2:00 *Lunch*

SESSION V: Alternatives Chairman: David P. Rall

2:00- 2:20 *Use and Replaceability of Polychlorinated Biphenyls*
Martin G. Broadhurst

2:20- 2:50 *Discussion*

2:50- 3:10 *Break*

SESSION VI: Where Do We Go From Here?

3:10- 3:30 *Norton Nelson*

3:30- 4:00 *Discussion*

4:00 *Adjourn*

MONS 201102

ABSTRACT for Chemistry - General Summary by Mr. J. William Cook

The general nature of the polychlorinated biphenyl compounds will be discussed. Emphasis will be given to the chemical properties which may have a bearing on the nature or combination of compounds found as residues in man's food supply.

MONS 201103

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH - NIEHS
P. O. Box 12233, Research Triangle Park, N. C. 27709

TO Participants in NIEHS Meeting on Polychlorinated Biphenyls (PCBs)

DATE December 22, 1971

FROM Editor, Conference Proceedings

SUBJECT Correction to Dr. Kuratsune's Presentation

1. Dr. Kuratsune has asked that attention be drawn to the inadvertent use of an incorrect datum in his presentation of Epidemiologic Study on Yusho.

2. The minimum dose of Kanechlor 400 consumed by a patient was 0.5g and not 0.2g as stated.

Douglas H. K. Lee, M.D., Ph.D.

MONS 201104

GESELLSCHAFT
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Reactions of PCB's upon UV-irradiation

K. Hustert, W. Klein, F. Korte

Definite PCB-isomers haven been synthesized according to literature (O. Hutzinger et al. 1971).

2,4,6,2',4',6'-Hexachlorobiphenyl has been irradiated with a mercury high pressure lamp using quartz filter.

Irradiation in solvents such as n-hexane, acetone, methanol and methanol/water resulted in a successive dechlorination with following intermediates: 2,4,6,2',4'-isomer, a trichloro- and a tetrachloro-isomer, 4,4'-dichlorobiphenyl, 4-monochlorobiphenyl and finally biphenyl. Apart from these, phenoles were formed too, especially when methanol was used as solvent.

Irradiation in perfluorinated hydrocarbons led mainly to isomerization and also to compounds with more than 6 chlorine atoms e.g. a heptachlorobiphenyl. This applies to the irradiation in solid state, too.

The authors were unable to attend the meeting, but thought this would be of interest.

MONS 201105