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.

MCNS 062983

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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 062984

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.

Effect of Aroclor 1254 on Ring Doves and Kestrels.

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and Stephen E. 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 annydrase 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 decrease 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 work : here

MONS 062986

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 ± 211.6 (481.2-1059.4)
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 C62988

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 occurwd 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.

MONS 062989

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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MCNS 062991

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 062997

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.

MONS 062998

2. Analytical epidemiologic studies.

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

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.

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 063001

At the present time our committee is composed of the immediate above nucleus. We are pleased to select Bill Papageorge as chairman for the proposed main committee.

Mr. Salazar, secretary for the Board of Directors at ANSI, is arranging to contact the numerous organizations listed, inviting them to send a representative to form a steering committee in May 1971, pursuant to formation of the prime committee at ANSI.

Through our active participation at SIGRE and the International Electrotechnical Commission, Mr. Raab of GE and the writer will pursue the international aspects of the PCB pollution problem.

P. G. Benignus

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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

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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.

PCB AND INTERACTIONS WITH INSECTICIDES

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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).

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.

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POLYCHLORINATED BIPHENYL INTERACTIONS WITH TISSUE CULTURE CELLS

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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.

The Sensitivity of Fish ATPases to Polychlorinated Biphenyls

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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.

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.

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.

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MONS 063014

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.

MCNS 063015